THE AMERICAN JOURNAL of

HUMAN GENETICS

VOLUME 6 March 1954 NUMBER 1

Published Quarterly by

THE AMERICAN SOCIETY OF HUMAN GENETICS

THE AMERICAN JOURNAL OF HUMAN GENETICS

is a quarterly record of research, review and bibliographic material relating to heredity in man, and to the applications of genetic principles in medicine, anthropology, psychology, and the social sciences. It is owned and controlled by the American Society of Human Genetics, and is edited by a staff appointed by its Board of Directors.

Subscription, per volume, $8.00 *Single numbers,* $2.50

THE American Journal of Human Genetics is published quarterly at Baltimore. Md. in March, June, September, and December. A volume will consist of four numbers, totaling approximately 400 pages. Subscription and other business communications should be addressed to the publishers, The American Society of Human Genetics, Mount Royal and Guilford Avenues. Baltimore 2. Maryland, or to the Treasurer, Dr. C. Nash Herndon, Department of Medical Genetics, Bowman Gray School of Medicine, Winston-Salem, North Carolina, U.S.A. Remittance for subscriptions from countries other than the United States must be payable in U. S. currency or its full equivalent. Checks or money orders should be made payable to the American Society of Human Genetics.

The American Journal of Human Genetics

Vol. 6 No. 1 *March 1954*

Maximum Likelihood Method for Estimation of Gene Frequencies from MNS Data[1]

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THE MNS series of genes is probably the second most useful known to anthropologists (Race and Sanger, 1950), and considerable bodies of MNS data are beginning to be available. To make such data most useful to anthropologists, reliable estimates of gene frequencies from them are required (Boyd, 1954b). Square root methods can be applied, as in the ABO case, but they suffer from the usual drawbacks of inefficient estimates (Stevens, 1938; Boyd, 1954b).

When tests are done, as is usual, with three sera, anti-M, anti-N, and anti-S, direct determination of the gene frequencies by gene counting is not possible, and in this the situation differs from the simpler MN case. Consequently some method of estimation is needed. Since the maximum likelihood method gives the estimates with smallest variance (Fisher, 1946, 1950; Mather, 1951; Stevens, 1938), this method has been applied to the MNS case. A preliminary note has already appeared (Boyd, 1954a).

There are four genes (or, if S be considered merely a closely linked gene at an adjacent locus, four chromosomes) involved in the MNS system. If we represent the gene frequencies by m_s, m_S, n_s, and n_S, and let G stand for the total number of subjects examined, the expected numbers in the six phenotypes will be

$$\begin{aligned} \mathrm{M} &= \mathrm{G}m^2_s \\ \mathrm{MS} &= \mathrm{G}(m^2_S + 2m_Sm_s) \\ \mathrm{MN} &= 2\mathrm{G}m_sn_s \\ \mathrm{MNS} &= 2\mathrm{G}(m_sn_S + m_Sn_s + m_Sn_S) \\ \mathrm{N} &= \mathrm{G}n^2_s \\ \mathrm{NS} &= \mathrm{G}(n^2_S + 2n_Sn_s). \end{aligned} \qquad [1]$$

Received November 30, 1953.

[1] The research reported in this paper was made possible in part by the use of equipment purchased by Boston University under Contract No. Nonr-492(01) with the Office of Naval Research.

The total frequency of the gene M (including gene M without S and gene M with S) can be obtained by gene counting just as in the simpler case, and is

$$m = \mathrm{M} + \mathrm{MS} + \mathrm{MN}/2 + \mathrm{MNS}/2$$

where M, MS, etc. represent the frequencies of the various classes in the sample. Similarly

$$n = \mathrm{N} + \mathrm{NS} + \mathrm{MN}/2 + \mathrm{MNS}/2.$$

These values m and n (which of course add to one) are given directly by the data, and no problem of estimation arises in determining them. This fact makes the subsequent calculations much simpler. We may write $m_S = m - m_s$, and $n_S = n - n_s$, and the only variables which remain to be estimated are m_s and n_s. To obtain maximum likelihood estimates, we have only to write out the likelihood expression, differentiate in terms of m_s and n_s, set these two derivatives equal to zero, and solve for m_s and n_s.

The likelihood equation is

$$\begin{aligned}\mathrm{L} = \mathrm{M}\log m_s^2 + \mathrm{MS}\log(m_S^2 + 2m_Sm_s) + \mathrm{MN}\log(2m_sn_s)\\ + \mathrm{MNS}\log 2(m_sn_S + m_Sn_s + m_Sn_S) + \mathrm{N}\log n_s^2,\\ + \mathrm{NS}\log(n_S^2 + 2n_Sn_s)\end{aligned}$$

or

$$\begin{aligned}\mathrm{L} = (2\mathrm{M} + \mathrm{MN})\log m_s + \mathrm{MS}\log m_S + \mathrm{MS}\log(m_S + 2m_s)\\ + (2\mathrm{N} + \mathrm{MN})\log n_s + \mathrm{MNS}\log(m_sn_S + m_Sn_s + m_Sn_S) \qquad [2]\\ + \mathrm{NS}\log n_S + \mathrm{NS}\log(n_S + 2n_s)\end{aligned}$$

where M, MS, etc., are the numbers of individuals observed to fall into the respective classes. If we now substitute $m_S = m - m_s$ and $n_S = n - n_s$ in this expression, and differentiate with respect to m_s and n_s, we obtain

$$\begin{aligned}\partial\mathrm{L}/\partial m_s = (2\mathrm{M} + \mathrm{MN})/m_s - 2\mathrm{MS}m_s/(m_S^2 + 2m_Sm_s)\\ - \mathrm{MNS}n_s/(m_sn_S + m_Sn_s + m_Sn_S)\end{aligned}$$

and [3]

$$\begin{aligned}\partial\mathrm{L}/\partial n_s = (2\mathrm{N} + \mathrm{MN})/n_s - 2\mathrm{NS}n_s/(n_S^2 + 2n_Sn_s)\\ - \mathrm{MNS}m_s/(m_sn_S + m_Sn_s + m_Sn_S)\end{aligned}$$

We are to find values of m_s and n_s which will make each of these expressions equal to zero.

The direct algebraic solution of these equations is not easy, but methods exist (Mather, 1951; Stevens, 1938) by which the proper values of m_s and n_s may be obtained by successive approximations. These methods (which are

similar to Newton's method of getting the numerical root of an equation) consist in substituting trial values of m_s and n_s into the equations, and, from the values then assumed by the equations [2], calculating corrections, which we may designate as δm_s and δn_s, from the relations[2]

$$\begin{aligned} -\partial^2 L/\partial m_s^2(\delta m_s) - \partial^2 L/\partial m_s \partial n_s(\delta n_s) &= \partial L/\partial m_s \\ -\partial^2 L/\partial m_s \partial n_s(\delta m_s) - \partial^2 L/\partial n_s^2(\delta n_s) &= \partial L/\partial n_s \end{aligned} \qquad [4]$$

where $\partial L/\partial m_s$ and $\partial L/\partial n_s$ are the values assumed by equations [3] when the trial values are substituted for m_s and n_s, and $\partial^2 L/\partial m^2$, and $\partial^2 L/\partial n^2$, are the second derivatives of the likelihood function.

Instead of working out the second derivatives explicitly, it proves simpler to compute them from the first derivatives of the expected values [1]. We may represent these derivatives generally as $\partial E/\partial x$. These derivatives are much simpler in form; for instance for the class M the derivative $\partial E/\partial m_s$ equals merely $2Gm_s$. The second derivatives may be calculated from the first derivatives by the relation

$$\partial^2 L/\partial x^2 = -\Sigma(1/E)(\partial E/\partial x)^2$$

where E represents the expected values, and x is any one parameter.

It is simple to derive and tabulate the derivatives of the expected frequencies from the genetical formulas [1]. They are:

PHENOTYPE CLASS	$\partial E/\partial m_s$	$\partial E/\partial n_s$
M	$2Gm_s$	0
MS	$-2Gm_s$	0
MN	$2Gn_s$	$2Gm_s$
MNS	$-2Gn_s$	$-2Gm_s$
N	0	$+2Gn_s$
NS	0	$-2Gn_s$

Substituting in these expressions the trial values we get the numerical values of $\partial E/\partial x$, dividing by E for each class, we get $(1/E)(\partial E/\partial x)$. By multiplying these together for each class, and adding, we get the second derivatives of the likelihood function. For instance, for the M class $\partial E/\partial m_s = 2Gm_s$ and, since $E = Gm_s^2$, $(1/E)(\partial E/\partial m_s) = 2/m_s$. The product is $2Gm_s \times 2/m_s = 4G$, and this is the contribution of this class to the value of $\partial^2 L/\partial m_s^2$. Since the derivative of E with respect to n_s is zero for this class, it makes no contribution to $\partial^2 L/n_s^2$. The cross derivative, $\partial^2 L/\partial m_s \partial n_s$, is obtained by computing the cross products $(1/E)(\partial E/\partial m_s)(\partial E/\partial n_s)$ and adding. The first two and last two classes make no contribution to this expression because of the zeros, but

[2] Obtained by the Taylor-Maclaurin expansion.

the two middle classes contribute the amounts $(2n_o/2m_on_o)(2Gm_o)$ and $(-2n_o/2(m_on_s + m_sn_o + m_sn_s)(-2Gm_o)$.

Having computed the second derivatives of the likelihood function, we may proceed to solve the two simultaneous equations [4] for δm_o and δn_o. Mather (1951) gives an example of a precisely similar solution for an ABO case.

Instead of doing this, however, it is preferable to solve, not equations [4], but the same set with different values for the constant terms, viz. the following

$$-\partial^2 L/\partial m_o^2 X_1 - \partial^2 L/\partial m_o \partial n_o Y_1 = 1$$
$$-\partial^2 L/\partial m_o \partial n_o X_1 - \partial^2 L/\partial n_o^2 Y_1 = 0$$

and [5]

$$-\partial^2 L/\partial m_o^2 X_2 - \partial^2 L/\partial m_o \partial n_o Y_2 = 0$$
$$-\partial^2 L/\partial m_o \partial n_o X_2 - \partial^2 L/\partial m_o^2 Y_2 = 1$$

After obtaining X_1, Y_1, X_2 and Y_2 from these equations, δm_o and δn_o may be obtained from the relations

$$\delta m_o = \partial L/\partial m_o X_1 + \partial L/\partial n_o X_2$$
$$\delta n_o = \partial L/\partial m_o Y_1 + \partial L/\partial n_o Y_2$$

The advantage of this procedure is that the variances of the estimates of m_o and n_o are given by the values of X_1 and Y_2, so the standard deviations of our estimates may be computed simply by taking the square roots of X_1 and Y_2. This avoids the solution of a new set of simultaneous equations to obtain the variances of m_o and n_o. The X_1, Y_1, etc. are the "c-multipliers" of Fisher's book (1950).

The reader already familiar with these methods of approximation will note that the above operations amount to the construction of the "information matrix" (Stevens, 1938; Mather, 1951), and its inversion to obtain the variance matrix

$$\begin{Bmatrix} X_1 & Y_1 \\ X_2 & Y_2 \end{Bmatrix}$$

It is customary to symbolize the negative second derivatives of the likelihood function by the letter I (standing for "information") because by definition the amount of information we possess about a variable m_o is given by the rate of change with m_o of the maximum likelihood equation [3], which means the second derivative of the likelihood function L. For cases in which the E values depend upon a single parameter, the variance of the estimate of the parameter is found as $V = 1/I$, or in words, the variance is the reciprocal of

the information. For cases in which more than one parameter is involved, the variance matrix is the inverse of the information matrix.

We have for the elements of the information matrix

$$\begin{aligned} I_{m_s m_s} &= \Sigma(1/E)(\partial E/\partial m_s)(\partial E/\partial m_s) \\ I_{n_s n_s} &= \Sigma(1/E)(\partial E/\partial n_s)(\partial E/\partial n_s) \quad \text{and} \\ I_{m_s n_s} &= \Sigma(1/E)(\partial E/\partial m_s)(\partial E/\partial n_s). \end{aligned} \qquad [6]$$

In the present instance (though apparently not in general) time and effort are saved by writing out in algebraic form the contributions of the various classes to the elements of the information matrix, and noting that they can be expressed in terms of the expected frequencies. The reader will be able to verify readily that

$$\begin{aligned} I_{m_s m_s} &= 4G[1 + (M)/(MS) + (N)/(MN) + (N)/(MNS)] \\ I_{n_s n_s} &= 4G[1 + (N)/(NS) + (M)/(MN) + (M)/(MNS)] \\ I_{m_s n_s} &= I_{n_s m_s} = 2G[1 + (MN)/(MNS)] \end{aligned}$$

where the expressions in parentheses represent the expected frequencies as calculated from [1], and G is the total number of persons examined.

Now, taking account of the fact that the solutions of two simultaneous equations can be written down immediately by inspection, we may reduce the computation of gene frequencies for MNS data by the method of maximum likelihood to very simple terms, involving no mention of matrices or other advanced mathematics, and no operation but simple arithmetic.

PRACTICAL COMPUTATION

Start with "consistent" estimates of m_s and n_s. By consistent estimates are meant any estimates which will approach the true values as the sample is indefinitely increased. For a first shot the square root estimates

$$\begin{aligned} m_s &= \sqrt{(M)} \\ n_s &= \sqrt{(N)} \end{aligned}$$

may be used, although the formulas of Mourant (see below) are better. Compute the gene counting estimates of total M and total N by the relations

$$\begin{aligned} m &= M + MS + MN/2 + MNS/2 \\ n &= N + NS + MN/2 + MNS/2 \end{aligned}$$

Estimate m_S as $m - m_s$ and n_S as $n - n_s$.

Using these frequencies, calculate the expected frequencies for each of the

six classes, from equations [1] (omitting G, which would divide out again anyway).

Calculate the two measures of the degree to which the trial gene frequencies differ from the maximum likelihood values, from equations [3], above.

Then calculate the three quantities $I_{m_s m_s}$, $I_{n_s n_s}$, and $I_{m_s n_s}$ by equations [6]. Let

$$V_{m_s m_s} = I_{n_s n_s}/(I_{m_s m_s} I_{n_s n_s} - I^2_{m_s n_s})$$

$$V_{n_s n_s} = I_{m_s m_s}/(I_{m_s m_s} I_{n_s n_s} - I^2_{m_s n_s})$$

$$V_{m_s n_s} = -I_{m_s n_s}/(I_{m_s m_s} I_{n_s n_s} - I^2_{m_s n_s}).$$

Then

$$\delta m_s = \partial L/\partial m_s (V_{m_s m_s}) + \partial L/\partial n_s (V_{m_s n_s})$$

$$\delta n_s = \partial L/\partial m_s (V_{m_s n_s}) + \partial L/\partial n_s (V_{n_s n_s})$$

The adjusted frequencies are

$$m'_s = m_s + \delta m_s, \qquad n'_s = n_s + \delta n_s, \qquad m'_S = m - m'_s, \qquad n'_S = n - n'_s.$$

The standard deviations of m_s and n_s are the square roots of the variances $V_{m_s m_s}$ and $V_{n_s n_s}$. I am indebted to Dr. Howard Levene for formulas for the variances of m_S and n_S. They are

$$V_{m_S} = V_{m_s m_s} + [(m - 2m_s)(1 - m)]/2G$$

$$V_{n_S} = V_{n_s n_s} + [(n - 2n_s)(1 - n)]/2G$$

The standard deviations of m_S and n_S are obtained by taking the square roots of these quantities.

This method may be illustrated by calculations of the gene frequencies from data obtained by me on 230 Bengalis in Dacca, East Pakistan. The crude data were

PHENOTYPE	NUMBER OBSERVED
M	35
MS	44
MN	47
MNS	62
N	21
NS	21
Total	230

For m we have $35/230 + 44/230 + 47/460 + 62/460 = 0.58043$, and for n, $21/230 + 21/230 + 47/460 + 62/460 = 0.41957$. Instead of square root values $m_s = \sqrt{(25/230)}$, $n_s = \sqrt{(21/230)}$, better values, from manipula-

tion of Mourant's equations (see below) were available. These were m_s = 0.37983, and n_s = 0.28954, giving m_S = 0.20060 and n_S = 0.13002. From these the expected frequencies were found to be

M	0.14427
MS	0.19263
MN	0.21996
MNS	0.26710
N	0.08384
NS	0.09220

From these values the quantities $I_{m_sm_s}$, $I_{n_sn_s}$ and $I_{m_sn_s}$ were calculated by equations [6]:

$$I_{m_sm_s} = 2248.46$$

$$I_{n_sn_s} = 2856.91$$

$$I_{m_sn_s} = 838.80$$

Then $V_{m_sm_s} = 2856.91/(2248.46 \times 2856.91 - 838.80^2) = 4.9945 \times 10^{-4}$, and similarly $V_{n_sn_s} = 3.9308 \times 10^{-4}$ and $V_{m_sn_s} = -1.4664 \times 10^{-4}$.

Substituting[3] the trial values of m_s and n_s into the two likelihood equations [3], we obtain

$$\partial L/\partial m_s = +0.090574, \quad \text{and} \quad \partial L/\partial n_s = -0.84908.$$

It is at this point in the calculations that accuracy in the calculations becomes especially important, for the quantities $\partial L/\partial m_s$ and $\partial L/\partial n_s$ are arrived at by adding several quantities, some of which are positive and some of which are negative, and the resulting sums are much smaller than the quantities which go to make them up. For this reason all factors should be known to two places of decimals (three in the case of data derived from series of the order of one thousand persons) *more* than are desired in the final results (Fisher, 1947). In general, in all such calculations, it is desirable to retain one or two places more than will be wanted at the end. Since the computations are done on calculating machines anyway, this adds very little to the work, insures accuracy, and facilitates checking.

[3] Another way (10) of obtaining the values of $\partial L/\partial m_s$ and $\partial L/\partial n_s$ consists in computing $\Sigma A(1/E)(\partial E/\partial m_s)$ and $\Sigma A(1/E)(\partial E/\partial n_s)$, where A signifies the observed number in a class, E the expected number, and Σ indicates summation for all classes. This method proves somewhat simpler when the number of parameters is large. It depends upon the identity

$$\partial L/\partial x = \partial/\partial x \Sigma A \log E = \Sigma(A/E)(\partial E/\partial x)$$

The question of the number of decimal places which should be reported has apparently troubled some workers in this field. It is hard to see anything wrong with the "one-third sigma rule" (Kelley, 1947), which states that there is serious doubt as to any magnitude as small as one third the standard error. The chances of a magnitude as great as this arising merely as a matter of chance are 74 in 100. Therefore, when we come to a decimal place which, by this test, could be uncertain by one or more units, there is no point in giving another place. Adherence to this rule in the present case means that three decimal places in the final results are all that are justified. More places have been given, partly to ensure accuracy in the computation, and partly for purposes of comparison of different methods of calculation.

Now we have

$\delta m_s = 0.090574 \times 4.9945 \times 10^{-4} + 0.84908 \times 1.4664 \times 10^{-4} = 0.00017$, and $\delta n_s = -0.00035$, giving us the corrected estimates $m'_s = 0.38000$ and $n'_s = 0.28920$. By subtracting these from m and n, we find $m'_S = 0.20043$ and $n'_S = 0.13037$.

The standard deviations are:

$$\sigma m_s = 0.02235$$

$$\sigma m_S = 0.01832$$

$$\sigma n_s = 0.01983$$

$$\sigma n_S = 0.01388$$

The corrections δm_s and δn_s were of the order of 1/50 of a standard error, and the estimates are now certainly sufficiently accurate. Otherwise the method could be applied again, using the adjusted values of m_s and n_s as the new trial values.

COMPARISON OF THE PRESENT METHOD WITH OTHERS

The gene frequencies can be estimated from MNS data by square root methods quite similar to those used in the ABO system. We have the formulas

$$m_s = \sqrt{\mathrm{M}}$$

$$m_S = \sqrt{(\mathrm{M} + \mathrm{MS})} - \sqrt{\mathrm{M}}$$

$$n_s = \sqrt{\mathrm{N}}$$

$$n_S = \sqrt{(\mathrm{N} + \mathrm{NS})} - \sqrt{\mathrm{N}}$$

In some cases the results obtained by the formulas might be accurate enough, but it will be noted that they make no use of the information contained in the MN and MNS classes, and in fact they are sometimes wide of

the mark. Also, of course, the variance of such square root methods is greater than that of maximum likelihood methods (Stevens, 1938; Fisher, 1950; Boyd, 1954b), and accordingly they are not efficient estimates.

Mourant (1953) has devised a method for the estimation of gene frequencies in the MNS system which, although it does not give the exact maximum likelihood estimates, is probably sufficiently close for many purposes. Dr. Mourant has kindly given me permission to present the method here in advance of its publication in his book.

Mourant computes m and n as usual. Then he obtains an estimate of m_s by making the product $m(\sqrt{[M/(M + MS)]})$ and of n_s by the product $n(\sqrt{[N/(N + NS)]})$. The sum of m_s and n_s ought to equal the frequency of s (the S negative gene), and the latter may be estimated by the square root method as $\sqrt{(M + MN + N)}$. If the sum of m_s and n_s is different from s, the values of m_s and n_s may be corrected by multiplying each by the quotient $s/(m_s + n_s)$. Alternatively one could obtain the frequency of S as $1 - s$, and correct $m_S = m - m_s$ and $n_S = n - n_s$ by multiplying each by the quotient $S/(m_S + n_S)$. Then the corrected estimates of m_s and n_s could be obtained by subtraction from m and n. This second procedure is the one favored by Mourant himself, and it will be called the original method here.

Applying these methods to the above data, we obtain

$$m_s = 0.58043 \times \sqrt{(35/79)} = 0.38634$$

$$n_s = 0.41957 \times \sqrt{(21/42)} = 0.29668.$$

The frequency of s is estimated as $\sqrt{((35 + 47 + 21)/230)} = 0.66920$. We may correct the estimates of m_s and n_s by multiplying by 0.66920/0.68302, obtaining $m'_s = 0.37852$, $n'_s = 0.29067$ (and the corresponding m_S and n_S) or we can subtract from m and n, obtaining $m_S = 0.19409$, $n_S = 0.12289$, and correct these by multiplying by $(1 - s)/(m_S + n_S)$ or 0.33081/0.31698, obtaining $m'_S = 0.20256$ and $n'_S = 0.12825$.

Results obtained by the crude square root method, the two Mourant methods, and the maximum likelihood methods obtained above, are shown in table 1.

TABLE 1. GENE FREQUENCIES CALCULATED BY VARIOUS METHODS

	SQUARE ROOT METHOD	MOURANT (ORIGINAL METHOD)	MOURANT (ALTERNATIVE METHOD)	MAXIMUM LIKELIHOOOD	STANDARD DEVIATION
m_S	0.19598	0.20256	0.20191	0.20043	0.01832
m_s	0.39009	0.37788	0.37852	0.38000	0.02235
n_S	0.12516	0.12825	0.12889	0.13037	0.01388
n_s	0.30217	0.29132	0.29067	0.28919	0.01983
χ^2	0.979	0.531	0.523	0.517	

It will be seen that in the present case the alternative Mourant method gives results agreeing somewhat better with the maximum likelihood values, but in certain other cases, as with the data calculated by Race and Sanger (1950), the original Mourant method seems to give better results than the alternative.

The values of χ^2 show that the estimates by either of Mourant's method fit the data quite well. In the present instance estimates by his method differ from the maximum likelihood values by about one-tenth of one standard error. For many purposes this would be close enough.

APPLICATION TO DATA OBTAINED WITH FOUR SERA

The above method may be applied, with appropriate modifications, to data obtained by testing with anti-M, anti-N, anti-S, and anti-*s*. In this case also simple gene counting is not possible, as one of the phenotypes contains two genotypes. Use of the present method presents no special difficulties.

SUMMARY

A maximum likelihood method is presented for calculating the gene frequencies from MNS data. By making use of the gene counting method to determine the total M and total N frequency in advance, the calculations are reduced to very simple form. The values so obtained are compared with those of the crude square root method and the approximate method devised by Mourant.

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On the Inheritance of the Antigen f of the Rh Complex[1]

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DETAILED analysis by Rosenfield, Vogel, Gibbel, Race and Sanger (1953) and Sanger, Race, Rosenfield, Vogel and Gibbel (1953) of the antibodies contained in the serum of a hemophilic patient who had had many transfusions revealed the presence of a previously unidentified antibody. This antibody was shown to react only with bloods derived from individuals bearing at least one chromosome with the antigens c and e of the Rh complex of agglutinogens, that is chromosomes of type *cde*, *cDe* or *cD^{u}e*. It was demonstrated (ibid) that the inheritance of the antigen detected by this antibody was not determined by any of the three known Rh loci $C - c$, $D - d$ and $E - e$. Because of the obvious close association of the antigen with the Rh locus and because of the demonstration that it was not inherited via any of the previously postulated Rh loci, the new antigen was named f. The antibody which identifies it is called anti-f.

Rosenfield and his colleagues assumed, as a working hypothesis, that f is inherited via a fourth pair of alleles ($F - f$) in the Rh complex, tightly linked to the other three loci. They postulated the existence of the antigen F with its detection awaiting the discovery of the appropriate antibody. This hypothesis permits the existence of such as yet unidentified chromosomes as *CDef*, *cDEf*, *cdeF*, etc.

Richardson Jones, Steinberg, Allen, Diamond and Kriete (1954) confirmed the existence of the anti-f antibody and the intimate association of the antigen f with the Rh system. They were sufficiently impressed, however, with the fact that all the available data agreed in showing that f is present only when *c* and *e* are on the same chromosome to believe that another hypothesis might be offered with equal validity to explain the inheritance of the f antigen. These authors suggested that f may be the result of a "position effect" such that when the genes *c* and *e* are together on the same chromosome (as in *cde*, *cDe* and *cD^{u}e*) the antigen f is present on the red cells. When the genes *c* and *e* are on opposite chromosomes as in *CDe/cDE* or when either (or both) is replaced by another allele the antigen f is not present.

A consequence of this hypothesis is that the f antigen should never occur

Received November 18, 1953.

[1] Supported in part by a grant #G3524 from The National Institutes of Health.

on a chromosome bearing the alleles *C* or *E*. Hence, chromosomes such as *CDef* or *cDEf* would not occur. Furthermore, the existence of F and its corresponding antibody is not necessary.

Evidence which would permit a choice between these hypotheses such as the discovery of chromosomes of type *CDef* or *cDEf* or the discovery of anti-F and a chromosome such as *cdeF* is not available. Some indirect evidence favoring the position effect hypothesis is available, however.

The present authors (1953) have studied the Rh blood groups of a family in which an allele of *c*, c^v (Race, Sanger and Lawler, 1948), in association with *e* was present in members of each of three generations.

Red blood cells derived from individuals having the allele c^v are agglutinated by all anti-c anti-sera and some, but not all, anti-C anti-sera. Consequently, the c^v antigen, as its symbol implies, is more closely related to the c antigen than it is to the C antigen. Nevertheless, according to the position effect hypothesis, f should not occur in association with c^v even though *e* is present on the same chromosome. The genotypes in the pedigree previously mentioned which are pertinent to our discussion are *cDE*/c^v*De* (one individual) and *CDe*/c^v*De* (in two of the former's sons). As predicted by the theory, the anti-f anti-serum failed to agglutinate the cells of any of these individuals. Sanger and colleagues (1953) have shown that the *cDE*^{u}, C^u*De* and C^u*De* chromosomes are f negative, thus lending further support to the prediction.

Other supporting evidence is derived from the statistical demonstration that f probably does not occur with *C* or *E*. This conclusion was referred to in an earlier paper (Richardson Jones and colleagues, 1954), but the detailed argument was not presented. The argument is as follows:

1. Chromosomes bearing the alleles *C* and *f* or *E* and *f* may be expected to occur if the inheritance of f is due to a fourth locus in the Rh complex. Examples of such chromosomes are *CDEf*, *CDef* and *cDEf*. If such chromosomes occur, the presence of anti-f in the anti-c and anti-e sera used for typing

TABLE 1. GENOTYPES INVOLVING THE CHROMOSOME *CDE* WHICH WOULD BE CLASSIFIED AS *CDe*/*cDE* IF ANTI-f IS PRESENT IN THE ANTI-c AND ANTI-e SERA USED FOR TYPING AND IF *f* OCCURS WITH *C* AND *E*

MOST PROBABLE GENOTYPE—ANTI-f NOT PRESENT		
CDE/*CDe*	*CDE*/*cDE*	*CDE*/*CDE*
	ACTUAL GENOTYPES	
CDEF/*CDef*	*CDEF*/*cDEf*	*CDEF*/*CdEf*
CDEF/*Cdef*	*CDEF*/*cdEf*	*CDEf*/*CDEF*
CDEf/*CDef*	*CDEf*/*cDEf*	*CDEf*/*CDEf*
CDEf/*CDeF*	*CDEf*/*cDEF*	*CDEf*/*CdEF*
CDEf/*CdeF*	*CDEf*/*cdEF*	*CDEf*/*CdEf*
CDEf/*Cdef*	*CDEf*/*cdEf*	
CDeF/*CdEf*	*cDEF*/*CdEf*	
CDef/*CdEF*	*cDEf*/*CdEF*	
CDef/*CdEf*	*cDEf*/*CdEf*	

could lead to errors. Table 1 illustrates the errors in typing which might occur in cases normally assigned most probable genotypes involving the chromosome *CDE*.

2. It is reasonable to expect anti-f to be present in anti-c and anti-e antisera. Persons who produce these antibodies are usually of the genotypes *CDe/CDe* and *cDE/cDE* respectively. Such persons are f negative and hence may produce anti-f when stimulated by cells derived from individuals bearing a chromosome such as *cde* or *cDe*. Cells from Rh negative individuals are the ones most often used to stimulate the production of anti-c or anti-e; hence, also anti-f.

We have been able to demonstrate the presence of anti-f in all five such anti-sera which we have tested.

3. It is possible to calculate the frequency with which *f* occurs with *C* or *E* if it is assumed that anti-f is present in the anti-c and anti-e sera used for typing. The derivation of the appropriate equations is as follows: (The derivation is given for *C*. It is, of course, identical for *E*.)

Possible combinations: CF Cf cF cf
Frequencies: u v w x

TRUE GENOTYPES	FREQUENCY	TYPED AS
CF/CF	u^2	*CC*
CF/Cf	$2uv$	*Cc*
Cf/Cf	v^2	*Cc*
CF/cF	$2uw$	*Cc*
CF/cf	$2ux$	*Cc*
Cf/cF	$2vw$	*Cc*
Cf/cf	$2vx$	*Cc*
cF/cF	w^2	*cc*
cF/cf	$2wx$	*cc*
cf/cf	x^2	*cc*

It is seen that all bloods of genotype *cc* are typed as such regardless of the presence of *f* and that only bloods of genotype *CF/CF* are typed as *CC*. All others are typed as *Cc*.

Then: $c = w + x = \sqrt{cc}$
$C = u + v = 1 - c$
$u = \sqrt{u^2} = \sqrt{\text{bloods typed } CC} = CF$
$v = C - u = Cf$

Estimated variances (V):

$$V_C = V_c = \frac{1 - c^2}{4N}; \qquad V_u = \frac{1 - u^2}{4N},$$

where N = number of bloods tested.

$$V_v = V_C + V_u - 2s_C s_u r_{Cu}, \quad \text{where} \quad r_{Cu} = \frac{\sqrt{c^2 u^2}}{\sqrt{(1 - c^2)(1 - u^2)}},$$

s = standard error and r is the correlation coefficient.

4. Race and Sanger (1950) have quoted data for 1798 English bloods tested by the five common Rh anti-sera. These data may be used to compute the frequency of occurrence of the *Cf* and *Ef* combinations. (It is assumed that all the anti-c and anti-e sera used contained anti-f.) In these data, the genotypes *cc* and *CC* were observed with frequencies of 0.3264 and 0.1841 respectively. The observed frequencies of *ee* and *EE* were 0.7065 and 0.0211 respectively.

The calculation of the frequency of *Cf* and *Ef* follows: Estimated Frequency of *Cf* and *Ef*: Data for English population, Table 27, Race and Sanger (1950). $N = 1798$ (where N equals the number of bloods tested).

1. *Cf:*

Observed Genotype	Observed Frequency
cc	.3264
CC	.1841

$$c = \sqrt{.3264} = .5713 = w + x$$
$$C = 1 - c = .4287 = u + v$$
$$CF = \sqrt{.1841} = .4291 = u$$
$$Cf = v = C - u = .4287 - .4291 \cong 0$$

v is clearly not significantly different from zero.

2. *Ef:*

Observed Genotype	Observed Frequency
ee	.7065
EE	.0211

$$e = \sqrt{.7065} = .8405 = w + x$$
$$E = 1 - e = .1595 = u + v$$
$$EF = \sqrt{.0211} = .1453 = u$$
$$Ef = v = E - u = .1595 - .1453 = .0142$$

The value of v is sufficiently great so that it may be significantly different from zero. Hence, it is necessary to calculate its variance (V_v) to test the probability that v is an estimate of $\bar{v} = 0$.

As shown above:

$$V_v = V_E + V_u - 2r_{Eu}s_E s_u$$

$$V_E = \frac{1 - e^2}{4N} = \frac{1 - 0.7065}{4(1798)} = 0.00004081$$

$$V_u = \frac{1 - u^2}{4N} = \frac{1 - 0.0211}{4(1798)} = 0.00013611$$

$$2s_E s_u = 2\sqrt{V_E V_u} = 0.00014906$$

$$r_{Eu} = \frac{\sqrt{e^2 u^2}}{\sqrt{(1 - e^2)(1 - u^2)}}$$

$$r_{Eu} = \frac{\sqrt{(.7065)(.0211)}}{\sqrt{(.2935)(.9789)}} = 0.2277$$

Hence: $V_v = 0.00014298$ and $s_v = .0120$
$v/s_v = .0142/.0120 = 1.183$
$P \cong 0.24$ that v is an estimate of $\bar{v} = 0$.

The calculations indicate that the assumption that the *Cf* and *Ef* combinations do not occur is consistent with the data.

Although the evidence presented above is in agreement with the requirements of the position effect hypothesis, it does not by any means render inadequate the hypothesis of a fourth locus. The demonstration of the failure of the f antigen to occur with alleles of *c* and of *e* rests on only one example for each of the alleles C^v, C^w, C^u and E^u. The calculation of the frequency with which *f* occurs on the same chromosome with *C* and *E* is based upon the reasonable but nevertheless unproven assumption that anti-f was present in the anti-c and anti-e sera used for testing and is at best only a probability. Furthermore, it must be pointed out that the data may be explained equally well by the multiple allele hypothesis favored by some workers in this country. The final choice of hypothesis must await further data.

SUMMARY

The evidence now available bearing on the correctness of the alternate hypotheses offered to explain the heredity of the Rh agglutinogen f is reviewed. It is concluded that this evidence tends to support, but does not prove, the position effect hypothesis.

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Hydrocephalus and Incomplete Fusion of Fetal Clefts. Report on a Kindred[1]

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THIS report concerns a kindred with hydrocephalus and several instances of incomplete fusion of fetal clefts which are normally closed during the first three embryonal months. An explanation of their causal genesis is outlined.

MATERIAL

The material consisted of a South Swedish kindred, A, with multiple developmental anomalies. The proband was one of 6,097 institutionalized mental defectives examined in a search for a specific form of mental deficiency (phenylpyruvic oligophrenia). The main observations are presented in the pedigree chart (fig. 1) and the case reports.

METHOD

Data for the investigation were obtained from parish registers, interviews with members of the kindred, hospital and maternity ward records. Thus information on eight generations was assembled. As far as possible, living members of the kindred were visited and examined for gross defects and mental deficiency. Most of the persons who had married into the kindred were also examined and of these all were found to be normal with one exception stated in the case reports. Data were collected on 407 individuals, of whom 221 were inmarried or related to the kindred through marriage.

CASE RECORDS

I:0 A woman (not in the pedigree chart), elder sister of I:2. When she was about 21 years old she was denoted as backwards in the church register.

II:21 A 53 year old man. He was said to have had St. Vitus Dance when at school. He would plunge his hands into his pockets so violently that they were destroyed. At the examination he was intellectually normal without malformations. He had involuntary jerky movements in his face accompanied by a noise like a hiccough. When he was tired these involuntary movements would occur every half minute. He said he was particularly disturbed by them when he had to be among people.

III:3 A 51 year old woman whose mother was examined and found to be normal and whose father was dead, but also said to have been normal. She was operated on for cleft palate at about seven years of age. At the examination she was found to be a little hard

Received October 23, 1953.

[1] This study was supported by a grant from the Reserve Fund for the Advancement of Medical Research, and by a grant to the Institute of Genetics from the Rockefeller Foundation.

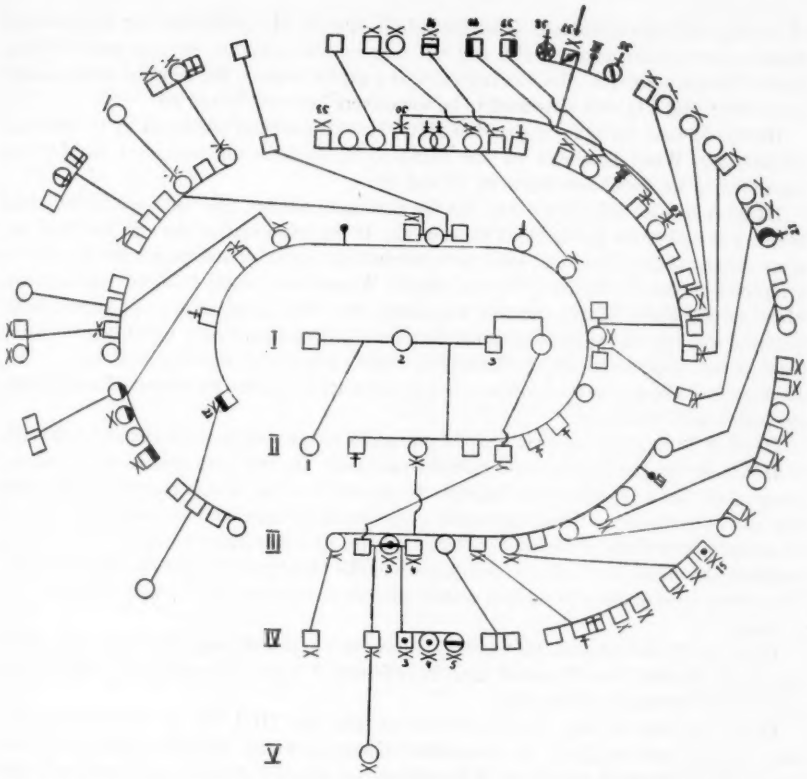
I
II
III
IV
V
Spina bifida
Hydrocephalus
Cleft palate
Hydrocephalus, spina bifida, and cheiloschisis
Encephalocele
Impaired hearing
Mental deficiency
Extrapyramidal lesion
Exstrophy of bladder
Umbilical hernia
Twins, probably monozygotic
Twins, probably dizygotic
Stillborn
Dead before 14 years of age
Abortion (Roman figure refers to month of pregnancy)
Examined by the author
Points to the probano

FIG. 1

of hearing and to have a speech defect (rhinolalia aperta). Her understanding of printed and spoken communications was faulty and her memory was defective. She was poor in finding sums of simple additions. She was employed as a garden worker. She kept her home in fairly good order. Her I.Q. was estimated to be somewhere between 70 and 80.

III:4 A 52 year old man, husband of III:3. He was a laborer employed by the town administration. When examined he was found to be intellectually backward, his I.Q. was estimated to be somewhere between 75 and 85.

III:20 A 26 year old woman. She was the proband's mother. She had been in fairly good health and had never had a blood transfusion. At the examination she was free from any gross abnormalities. Head and spine were normal at inspection and palpation. She had no symptoms or signs of disease in internal organs. Wassermann reaction (blood) was negative; Meinicke and Kahn (blood) negative. Cutaneous test with toxoplasmin was positive; Sabin-Feldman dye test was positive in serum dilution $\frac{1}{10}$ (50% hemolysis). Complement fixation reaction was negative in serum dilution $\frac{1}{5}$ (100% hemolysis). Blood groups were O, MN, Rh-negative. There were no symptoms or signs of central nervous disturbance. Intellectually, she was a good average.

III:30 A 29 year old man. He was the proband's father and the husband and first cousin of III:20. By occupation he was a railroad yardsman. He had been treated for pulmonary tuberculosis but was, otherwise, healthy. At the examination he was found to be free from any gross abnormalities. Head and spine were normal at inspection and palpation. He had no symptoms or signs of disease in internal organs. The Wassermann reaction (blood) was negative; Meinicke and Kahn (blood) negative. His blood groups were O, M, Rh-positive. There were no symptoms or signs of central nervous disturbance. His intelligence was a good average.

III:33 A 22 year old man. He was said to have had a notably large head as a child. When examined, the head was of normal size, circumference 585 mm. No anomalies were observed and he was normal intellectually.

IV:3 A 13 year old boy who was the son of III:3 and III:4. He was late in learning to talk. At eight years of age he was incontinent of urine and feces. It was not possible to teach him reading, writing or arithmetic at the elementary school and he was confined to a school-home for mental defectives. Upon examination he was found to have a somewhat high palate but no physical abnormalities. He did not know his date of birth, got right and left mixed, made grimaces and would suddenly clash his hands together. His conduct, on the whole, was orderly. His I.Q. was estimated to be about 55–65.

IV:4 An 11 year old girl. She was the eldest daughter of III:3 and III:4. At 10 years of age she was incontinent of urine and feces. She was unable to learn to count at school but could read fairly well. At the examination she was found to be physically normal with a fairly high palate. She did not know 3×4. She knew the difference between right and left. Her conduct was, on the whole, orderly. She consistently failed in all subjects while attending a normal school and was then enrolled in the school's classes for retarded children, but failed in this work as well. She gave the general impression of having an I.Q. between 65 and 75.

IV:5 A 7 year old girl. She was the youngest daughter of III:3 and III:4. At 2 years of age she was operated on for palatoschisis. Her marks after her first year at school were low average. On examination, it was noted that she had no speech defect. Her ability to draw, count and write seemed to warrant an estimate of her I.Q. to about 90. By general impression she was the brightest member of the family.

IV:11 A boy (not in the pedigree chart), grandson of II:1. His parents were visited by me, they were normal. He was the seventh of eight sibs. When he was five years old roentgeno-

logical examination revealed transposition of the viscera. At 10 years he died of lymphocytic meningitis.

IV:15 A 19 year old man. An undated examination report, probably early in his seventh year, described him as a hydrocephalic imbecile. When he was seen by another doctor at 7½ years of age his bodily development was described as normal. At school, he was ineducable. His mental quotient was 73 at 7 years, 57 at 8 years, 47 at 10 years and 44 at 13 years. When confined to a school-home for mental defectives he was unable to benefit by the theoretical education. His speech was broken with stuttering and was indistinct. Examination affirmed his imbecility and revealed a disproportionally big head with a circumference of 620 mm, length 202 mm, breadth 166 mm, and height 157 mm.

IV:27 A girl, the daughter of normal parents. She was born in face presentation and weighed at birth 3,800 Gm. According to hospital records, she was born with a severely misshaped head. She died three days after birth. Her father was told by the physician who handled the delivery that she was born with her brain uncovered and that she had a cerebral hernia.

IV:35 A girl who was the eldest child of III:20 and III:30. Her mother was well during pregnancy. At delivery, there was abundant amnionic fluid. She weighed at birth 2,700 Gm. Head circumference was 33 cm, shoulder circumference 32 cm, and body length was 44 cm. She had spina bifida and myelomeningocele and died twelve days after birth.

IV:37 A 4 year old boy, the proband. He was the second child of III:20 and III:30. Before the pregnancy, his mother had a spontaneous abortion in the third month and had no menstruation before the proband was born 11 months later. She had anemia during pregnancy. At the delivery there was abundant amnionic fluid. The proband weighed at birth 3,600 Gm., was 49 cm. long and had a head circumference of 37 cm. There was no icterus. Eleven hours after birth the child was soporous and during the following days he had to be treated with oxygen. He had a tense fontanel and a leftsided facialis paresis. He vomited frequently. When five days old he was admitted to a children's hospital. His head then appeared to be a little enlarged and had a circumference of 38 cm. The anterior fontanel was tense and there was a sutur diastasis. He had inward strabismus but no other pareses. During the two days he remained at the hospital there was no note made on icterus. When he was 2½ months old the parents observed that his head was larger than normal. He was seen again at the children's hospital when he was 6 months old. His head circumference was then 57 cm. He had a large sutur diastasis, protruding veins of the scalp, and depressed orbital roofs. At the age of 8 months he was admitted to the Neurosurgical Clinic of the University of Lund. His head circumference was then 62 cm. The anterior fontanel was tense, 16 x 18 cm., and the posterior fontanel was 5 x 3 cm. The left corner of his mouth was lagging a little. The eye fundi were normal. Ventriculography disclosed a huge hydrocephalus with only a very thin layer of brain tissue.

When examined at 4 years of age he had a head circumference of 69 cm., and a body length of 79 cm. The length of the head was 210 mm., breadth 212 mm., height 191 mm. Width of the anterior fontanel was 10 x 10 cm. The palate was fairly high. He had paretic legs, moved his right arm very little and grasped for things with his left hand. The feet were in reversible equinus position. Chest and abdominal organs were normal. The testes were not palpable. He was incontinent of urine and feces. The Wassermann reaction (blood) was negative; Meinicke and Kahn reactions (blood) also were negative. Toxoplasmin cutaneous test was negative. Sabin-Feldman dye test negative, and the complement fixation reaction also was negative. His blood groups were O, M, Rh-positive. He smiled when played with, could not talk, did not react when spoken to, but recognized people who handled him daily.

When re-examined at 5½ years, he was in essentially the same condition; could not sit and could not balance his very big head.

IV:38 A girl, the youngest child of III:20 and III:30. Her mother was mentally depressed during the pregnancy, but otherwise well. The child was born in breech presentation. She weighed at birth 2,400 Gm., measured 49 cm. long and had a head circumference of 35 cm. She had hydrocephalus, myelomeningocele, cheiloschisis and died five days after birth.

IV:39 A 3 year old boy born by normal delivery. At birth he weighed 2,500 Gm., was 47 cm. long, had a head circumference of 33 cm. and a shoulder circumference of 30 cm. In the hospital file it was recorded that the newborn boy had an undeveloped penis and exstrophy of the urinary bladder. When examined he had been operated on and the defect of the abdominal wall was closed. The urethra opened on the proximal part of the upper surface of the body of the penis. Mentally he was normal.

IV:40 A 3 year old boy. The parents were examined: normal. He had an umbilical hernia, which was observed when he left the maternity ward. When he was 12½ months old the hernial sack admitted one finger. He was operated on with a good result. On examination he was observed to be well built and mentally normal.

IV:41 A 4 year old boy. The parents were found normal upon examination. The boy was born with palatoschisis. At the age of 2 years he was admitted to the Otiatric Clinic, University of Lund, and was observed to have a broad cleft in the palate. When examined at 4½ years of age he had a broad head which looked as if pressed together a little from above. His palatal defect had been operated on. He had ptosis of the left eye and positive Babinski on the left side. Intellectually, he seemed to be normally developed.

DISCUSSION

In the kindred presented here the increased frequency of developmental defects can scarcely be explained as a coincidence. Within the South Swedish population from which this kindred is derived, Böök (1951) found the following frequencies of the pertinent anomalies among new-born children. The figures state the incidence per 10,000.

Cheiloschisis	3.2 ± 0.9
Ectopia vesicae urinariae	0.5
Encephalocele	0.5
Isolated palatoschisis	3.9 ± 0.9
Hernia umbilicalis	2.0 ± 0.7
Hydrocephalus	10.0 ± 1.5
Meningocele	0.9
Myelomeningocele	2.3 ± 0.7
Spina bifida	10.7 ± 1.6

In determining whether the fusion anomalies observed in the proband's generation of this kindred occurred by coincidence, the figures from Böök's series could be adopted. Umbilical hernia, however, of the socalled acquired type occurs in a higher frequency than the congenital type which probably predominates in Böök's material. In a series of 1,526 infants examined at the Children's Health Center of the Pediatric Clinic in Karlstad, Karlström (1947) found umbilical hernia in 11.1%. Slightly less than one third of these patients belonged to a group in which the neck of the hernial sack admitted

of the passage of at least a finger tip. In this same group Karlström observed that 72.2 ± 10.6 per cent of the untreated patients were healed before the age of 12 months. In the case of IV:40 in the kindred reported here, the neck of the hernial sack admitted one finger, when the patient was 12 months old. The frequency of hernias of this type could be calculated to be about $11.1/100 \cdot 1/3 \cdot 27.8/100 = 1/100$ appr. To avoid stretching the quoted figures too far in favor of the hypothesis of nonrandomness, the figure of 11.1 per cent will be used in the following calculation.

In the following calculation 49 normal individuals of generation IV, two with isolated cleft palate, one with exstrophy of bladder, one with encephalocele, one with umbilical hernia, and one with hydrocephalus are represented by the frequency figures mentioned previously. This gives the chance figure of the observed distribution of anomalies within a sample of 55 individuals from a population including stillborn children. $55!/49!\cdot 2!\cdot[1 - (3.9/10000) - (0.5/10000) - (0.5/10000) - (11.1/100) - (10/10000)]^{49}\cdot(3.9/10000)^2\cdot 0.5/10000\cdot 0.5/10000\cdot 11.1/100\cdot 10/10000 = 1/10^{12}$.

In the series of 6,097 mental defectives 30 were hydrocephalic, probably of the congenital type, i.e. 50.8/10000. In 29 other cases hydrocephalus of the congenital type could not be excluded. The frequency could be 96.8/10000 or even higher as hydrocephalus was not completely registered. No other case of exstrophy of the bladder occurred in the series. Harelip with or without cleft palate and isolated cleft palate occurred in 38 cases and spina bifida was registered in 5 of the hydrocephalus cases; 2 cases of encephalocele were recorded.

A calculation with frequencies of hydrocephalus 10 times higher and isolated cleft palate 20 times higher than among newborn children in the general population, and with the observed frequency of 3.3/10000 for encephalocele would not yield figures of an essentially higher degree of magnitude. If it were assumed that these higher frequency figures were applicable also on the sibs and cousins of the index cases, then a chance figure of 1.3×10^{-8} could be calculated. This figure would represent the chance of obtaining, within such a subpopulation with higher frequencies of hydrocephalus and fusion defects, a sample of 55 individuals where such defects occurred in the frequencies actually observed if no common etiologic factor were at work. Here the sibship with the proband is still calculated as represented by only one individual.

To this figure could be added the chances of the defects occurring in other combinations and in still greater number. As far as the common anomaly of umbilical hernia is omitted the chance figure will not be great enough to impair the conclusion: that the coincidence of hydrocephalus and fusion defects in this group of individuals with a common ancestry is not of random occurrence. It may, then, be assumed that the common cause of the various malformations is of hereditary kind.

TABLE 1. FREQUENCIES OF INDIVIDUALS WITH HYDROCEPHALUS AND ASSOCIATED ANOMALIES AMONG DESCENDANTS FROM AN *IICc* x *Iicc* MATING

GENERATION	NUMBER OF ANOMALOUS INDIVIDUALS		NUMBER OF INDIVIDUALS
	Observed	Expected	
II	0	0	14
III	1	2.9	46
IV	5*	4.2	54*

* The proband and his sibs excluded.

The most simple mode of inheritance which fits the observed frequencies is dominance for a gene C causing fusion anomalies and hydrocephalus, and epistasis of an inhibiting gene I. Abnormal individuals are iiCC or iiCc. If I:2 is IICc and I:3 is Iicc, then their offspring, mating iicc individuals, will have abnormal descendants in the frequencies demonstrated in table 1.

The calculated chance for anomalous offspring in generation IV by exogamy in generation III is approximately 1/13 and by cousin marriage 1/10, provided that all categories occurring within the kindred have the same chance of reproduction. The chance of abnormality in a particular child from a first cousin marriage between normal individuals within the kindred is 1/20 and the chance of all three children being abnormal in such a marriage is $(1/20)^3 = 1/8000$. The actual observation of such a family is, in fact, unique in the series of 6,097 mental defectives, where information on relatives included as a rule parents, very often sibs and not seldom, cousins, uncles and aunts.

This is an attempt to explain by deductive reasoning the occurrence within this kindred of hydrocephalus and fusion anomalies in a frequency too high to be fortuitous. Probably the suggested causal genesis is of relatively rare occurrence.

Major central-neural malformations and extra-neural fusion anomalies are not, as a rule, inherited interchangeably. This has been demonstrated by investigations in which adequate control series and frequency figures from the populations were used (Fogh-Andersen, 1943, Record and McKeown, 1950).

There is evidence, however, that hydrocephalus and fusion anomalies sometimes have a common cause. Record and McKeown (1949) observed association in one individual of hydrocephalus with spina bifida, encephalocele with spina bifida, and hydrocephalus with encephalocele and spina bifida in frequencies far higher than expected in comparison to the frequency figures from 158,307 births. Among 471 individuals with spina bifida and 59 with cranium bifidum Fisher, Uihlein and Keith (1952) observed hydrocephalus in 150 cases. Fogh-Andersen (1943) observed that harelip and cleft palate were associated with other anomalies in one individual in 10 per cent or more

of the cases. It seems likely, from such observations, that noxae causing hydrocephalus as well as agents inducing harelip and cleft palate can produce lesions of varying localisations or that, in some cases, there is a cause and effect association between different anomalies.

Many observations of extra-neural fusion anomalies associated with central-neural abnormalities in one individual have been published. Record and McKeown (1949) observed harelip with or without cleft palate in three patients and exomphalos in two out of a series of 389 individuals with spina bifida. Fogh-Andersen (1943) observed that encephalocele occurred in one of 215 probands with isolated cleft palate. In a series of 76 newborn with harelip in combination with cleft palate he encountered one individual with encephalocele and oblique cleft of face, two with cranioschisis, and three with hydrocephalus. Such observations are suggestive of one agent sometimes being capable of causing central-neural and extra-neural fusion anomalies and hydrocephalus.

The nature of agents with such effects might be varied. Warkany, Nelson and Schraffenberger (1943) got offspring with cleft palate and other defects from female rats reared on a deficient diet. Baxter and Fraser (1950) obtained cleft palate and cerebral hypoplasia in young from female mice treated with cortisone during the stage of pregnancy corresponding to the time of closure of the naso-maxillary fissure. Spina bifida was also observed in the offspring of treated mice (Fraser and Fainstat, 1951). Cleft palate was produced in the same strains by treating pregnant females with 17-hydroxycorticosterone acetate (compound F), (Kalter and Fraser, 1952). Offspring from mice subjected during pregnancy to periods of anoxia had anencephaly or cleft palate (Ingalls, Curley and Prindle, 1950).

Gillman, Gilbert, Gillman and Spence (1948) treated female rats with trypan blue before conception and during pregnancy. The litters contained individuals with hydrocephalus, spina bifida, eye defects in a high frequency and, in a less high frequency, tail defects, meningocele, harelip and cleft palate, cranioschisis, ear defects, imperforate anus, club foot and dislocations of the fore and hind limbs, and umbilical hernia. Serial sections of the brain in hydrocephalic animals revealed a completely communicating ventricular system, the hydrocephalus was interpreted as a result of a disturbed secretion-absorption rate of the cerebrospinal fluid. Hogan, O'Dell and Whitley (1950) obtained hydrocephalus in less than one percent in litters from female rats supplied with a diet defective in ascorbic acid, niacin, folic acid and vitamin B_{12}. When folic acid antagonist was added to this diet, the incidence of hydrocephalus rose to 20 per cent.

From such experiences it may be concluded that various non-genic agents can produce hydrocephalus and that the same agents can sometimes interfere with the formation of structures where the closure of fetal clefts is involved.

A gene with an effect equivalent to that of such agents has been assumed to be responsible for the major congenital defects observed within the A kindred. The abnormalities which have been discussed here seem to belong to a definite class; numerous clinical and experimental data point to a common genesis.

Some anomalies recorded in the pedigree chart and the case reports have been left out of the discussion as they could not be referred to this category of related disturbances. In the case of IV:15 and III:33 this was because of incomplete clinical data; arrested hydrocephalus could not be excluded. In the calculations they are entered as normal. The stillborn children had not undergone postmortem examination, but from the scanty hospital data on record it was possible to conclude that they were most probably free from visible malformations. The sibs of the proband's grandfathers who had died as children were said to have been normal, the causes of death were scarlet fever in one case, drowning by accident in one, and unknown in three instances.

SUMMARY

In a South Swedish family group, individuals with hydrocephalus and incomplete fusion of fetal clefts occurred in such a high frequency that they were probably of a common hereditary origin.

The simplest mode of inheritance which fits the observed frequencies seems to be dominance of a gene with variable expressivity and epistasis of an inhibiting gene, responsible for an incomplete penetrance.

Such an interpretation would be compatible with observations by various investigators that hydrocephalus and fusion anomalies occur in the same individual with appreciable frequency. It seems also to be in agreement with experimental observations of non-genic agents causing hydrocephalus interchangeably with fusion anomalies.

ACKNOWLEDGMENT

I am grateful to Professors J. B. S. Haldane and Carl-Erik Quensel for discussion of an earlier form of the manuscript.

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Populations of Hybrid Origin as Source Material for the Detection of Linkage

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A CORRELATION in the occurrence of two genetic traits within a population is usually interpreted as evidence against, rather than for linkage. Linkage results in correlations within families, but in opposite types of correlation from one family to another, the type of correlation within a particular family depending upon whether most of the chromosomes are in the coupling or repulsion phase. Thus opposite types of correlation within different families cancel correlations within the population as a whole.

This corollary is derived from the observation that mutations are recurrent and that they appear to occur independently. Regardless of whether linked or not, genes at different loci tend to occur independently of each other within panmictic populations. The fact that the Rh antigens, C, D, and E do not occur independently of each other (Rife, 1948) casts doubt on Fisher's belief that they are linked (Fisher, 1947) unless they are so closely linked that crossing over never occurs. Pleiotropy provides a reasonable interpretation of consistent correlations between genetic traits in all population. If Wiener (1946) is correct in assuming that a single series of multiple alleles is responsible for the Rh variations, some members of the series are pleiotropic, as they produce two or more antigens.

Relatively new populations of hybrid origin provide an exception to the foregoing rule. Under specific circumstances correlations within them may be indicative of linkage. This paper is concerned with the nature of these circumstances, and the report of an investigation of two hybrid populations which revealed associations suggestive of autosomal linkage.

CORRELATIONS WITHIN HYBRID POPULATIONS

Members of an allelic series attain genetic equilibrium with only a single generation of random mating. (Hardy, 1908, Weinberg, 1908). This does not hold true for the relationships between the members of different sets of alleles. (Robins, 1918, Haldane, 1925, Li, 1948). The rate at which equilibrium is approached depends solely upon the percentage of crossing over. Within a hybrid population the deviation from equilibrium is reduced by half each generation if the loci are not linked, or if linked by the amount of crossing over between the two loci. The initial degree of deviation from equilibrium depends

Received July 25, 1953.

upon relative sizes of the parent populations, and the differences in frequencies of the genes under consideration. In situations where the parent populations are of opposite homozygous genotypes (*AABB* × *aabb*, or *AAbb* × *aaBB*) the rate of approach to equilibrium may be readily calculated. The proportion of recombination gametes in any particular generation = $1 - [x^2 + y^2 + 2xy(1-c)^n]$ where x and y represent the relative proportions of the two parent populations, c = percentage of crossovers, and n = number of generations since hybridization. Figure 1 illustrates the rate of equilibration for six generations where x and y each = .5; for no linkage (50% crossovers), 20, 10, and 5% crossovers. The deviations from equilibrium are represented by the heights of the lines above the base.

Non-linked genes approximate equilibrium after six generations, whereas those linked with 5% crossovers show only about 10% recombinations over the same period. Over a hundred generations would be required for genes linked with 1% crossovers to approximate equilibrium. It is evident that closely linked genes may manifest correlations within hybrid population, many generations after non-linked genes have attained approximate equilibrium. Thus if a marked correlation is apparent between two genetic traits within a hybrid population, whereas no correlation exists between a third genetic trait and either of the other two, the correlation strongly suggests that the first two traits are conditioned by linked genes, while the third is probably due to genes

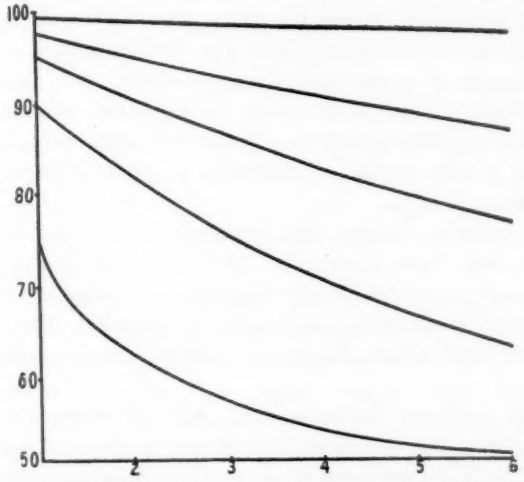

FIG. 1. Graphic representation of the rate at which two pairs of alleles approach genetic equilibrium over a period of six generations, in a population of hybrid origin. The percentages of non-recombination gametes are represented on the vertical axis, the number of generations on the horizontal axis. Rates of equilibration are shown for no linkage, and linkage with 20%, 10%, 5%, and 1% crossovers. The parent populations were of equal size (x = .5, y = .5).

on another chromosome. Pleiotropy may also result in a correlation in the incidence of genetic traits, but if this be the cause of the association, we should expect similar correlations in all populations, whereas linkage would result in no correlation within stable populations, or perhaps the opposite type of correlation within other hybrid populations.

If certain conditions are met a correlation between two out of three genetic traits may be considered as evidence for autosomal linkage. They are as follows: the population in which the correlation occurs must be of hybrid origin, the parent populations must have been characterized by marked differences in the frequencies of all of the genes under consideration, at least one of the traits showing the correlation must be of no selective importance; and the correlation must be absent in old established populations. It is difficult to conceive of any cause, aside from linkage, which would satisfy these conditions.

THE HYBRID POPULATIONS AND THEIR ORIGINS

Two populations of hybrid origin were investigated, both of which are of Negro-Caucasian descent. One population consisted of 100 northern Sudanese, the other of American Negroes.

The northern Sudanese are principally a mixture of Arabs and Negroes with some admixture of Hamitic peoples. Arabs have migrated to the northern Sudan from Arabia across the Red Sea and from Egypt as far back as the seventh century A.D. Until recent times they captured Negroes from the southern Sudan and other regions for slaves, and frequently married them. Although the northern Sudanese of today are Mohammedans and are Arabic in culture, evidences of considerable Negro ancestry are readily apparent.

Data were obtained from students at the Egyptian secondary school in Khartoum.[1] These students come from all over the northern Sudan, and would seem to provide a wide sample of unrelated northern Sudanese. All of the students are male.

The data on American Negroes were obtained over a period of years from students at the Ohio State University. Although 99 individuals have been included in the study, complete data for the present investigation were available for only 35 of them. Both males and females are included. American Negroes are essentially of West African Negro and northwestern European or British descent.

Although both northern Sudanese and American Negroes are of mixed Negro-Caucasian descent, the Negro and White ancestors of each are somewhat different. The Negro ancestors of the northern Sudanese were presumably

[1] The data were collected during February, 1952, by an expedition consisting of Mr. Martin Johnson, Mr. Robert Murphy, Dr. and Mrs. E. T. Bullard, Mr. Rafia Bouymi, and the author. Grateful acknowledgement is due Dr. Roha, supervisor of Egyptian education in the Sudan, for his cooperation in the project.

Nilotes and other Sudanese for the most part, whereas the ancestors of the American Negroes were largely West African. The White ancestors of the Sudanese were mostly semitic (Arab), whereas those of the American Negroes were northwestern European and British.

TYPES AND COLLECTION OF DATA

Data collected from the northern Sudanese include kodachrome photographs of heads, taste reaction to phenylthio-carbamide, and hand prints. The photographs were taken out-of-doors in the shade, over a two hour period. Conditions were maintained as uniform as possible throughout the procedure. One member of the expedition recorded his judgment as to shade of pigmentation for each individual. The author classified the photographs as to "light" and "dark" according to his judgment, before referring to the observations noted by the observer at the time the photographs were taken. Agreement in judgment was noted in 95% of cases. The observer who recorded his judgments used 5 shades, and the author found that his "darks" correspond to the two darkest shades, and his "lights" to the three lightest shades recorded by the observer. The observer obtained the following distribution beginning with the darkest shade: 23, 17, 53, 6, 1. None of the darkest shade are as dark as the pictures of over 300 Nilotic Negroes taken at a later date, and only the one in the extreme light class could be assumed to be of unmixed White ancestry with reasonable assurance. All of the subjects possessed black or very dark brown hair, ranging from curly to woolly in form. The "dark" northern Sudanese referred to henceforth include the two darkest classes of the observer, the "light" Sudanese to the three lightest classes, a total 40 "dark", and 60 "light" Sudanese.

Taste reaction to phenylthio-carbamide was determined by placing a small amount of the substance on the back of the tongue and noting whether the subject detected a distinct taste after a minute. Prints were obtained of entire palms and the fingertips. The taste reactions and hand prints were also obtained for American Negroes.

Black and white photographs of both front and profile head views were available for 44 American Negroes. These were taken under uniform conditions by the Department of Photography of the Ohio State University. The photographs were arranged independently into "dark," "light," and "doubtful" by four individuals who were unfamiliar with the people who had been photographed. Agreement was reached on 19 "dark," 16 "light," and 9 "doubtful."

Skin pigmentation, dermatoglyphics, and tasting ability fulfill the requirements for testing for linkage in hybrid populations. Each show marked differences in their frequencies in the parent populations, and neither dermatoglyphics or tasting ability are of any selective importance. The three traits occur independently of each other in panmictic populations. Tasting ability

appears to be determined by a single pair of alleles, dermatoglyphics include several types of variations due to multiple genes, and the differences in pigmentation between Negroes and Caucasians appear to be due to at least two pairs of alleles (Davenport, 1913).

Data from various investigators indicate that most African Negroes have significantly higher frequencies of tasters than do Caucasians (Lee, 1934, Parr 1934, Rife, 1953a).

Palmar dermatoglyphics likewise occur with different frequencies among Negroes and Caucasians, Negroes being characterized by higher frequencies of patterns in the second and fourth interdigital areas, and lower frequencies in the third interdigital and hypothenar areas (Pons, 1952).

The incidences of whorls on fingertips, however, do not manifest as clear cut differences between Negroes and Caucasians in general, as they do among both Negro and Caucasian populations. Some West African Negroes have approximately 40% whorls, whereas Nilotic Negroes possess between 25% and 30% whorls. Among Caucasians northwestern Europeans show 25% to 30% whorls, whereas Middle Eastern peoples show approximately 40% whorls (Rife, 1953b).

The ABO blood groups occur with somewhat similar frequencies among African Negroes, northern Sudanese, Ethiopians, and Bedouins (Rife, 1953a). Data on associations between blood groups and pigmentation in northern Sudanese would thus shed little light on linkage relationships and were not included in this investigation. The situation is different among American Negroes, as West Africans and White Americans show highly significant differences (table 5). Group A occurs approximately twice as frequently among Whites as among Negroes, whereas B occurs approximately twice as frequently among African Negroes as among American Whites.

RESULTS

Tests were made for associations between the occurrence of patterns on each of the five palmar areas with pigmentation, with ability to taste, and of pigmentation with tasting ability. The incidence of patterns in the second interdigital area revealed a highly significant association with pigmentation the "dark" showing much higher frequency of patterns than "light" (table 1).

TABLE 1. ASSOCIATIONS BETWEEN PIGMENTATION AND PALM PATTERNS IN SECOND INTERDIGITAL AREA

	DARK		LIGHT	
	Pattern	No pattern	Pattern	No pattern
100 Northern Sudanese	11	29	4	56
35 American Negroes	3	16	0	16
Total	14	45	4	72

$X^2 = 9.46$, df = 1, $p < .01$

TABLE 2. ASSOCIATIONS BETWEEN PIGMENTATION AND ABILITY TO TASTE PHENYLTHIOCARBAMIDE

	DARK		LIGHT	
	Tasters	Non-tasters	Tasters	Non-tasters
100 Northern Sudanese	38	2	56	4
27 American Negroes	12	5	10	0
Total	50	7	66	4

$X^2 = 1.93$, df = 1, p between .70 and .50

The higher incidence of patterns among "dark" is apparent among both Sudanese and American Negroes. No significant associations were found between pattern occurrence and tasting ability, or between tasting ability and pigmentation (tables 2 and 3). The high association between patterns in the second interdigital area and dark pigmentation, and the absence of associations between taste and either pattern of pigmentation strongly suggests linkage between genes responsible for patterns and pigmentation. Pleiotropy seems to be ruled out, as patterns occur among both parent populations, although with greater frequencies among Negroes.

No significant correlations were found between pigmentation or tasting ability and the occurence of patterns in any of the other areas. Whorls on fingertips, however, show a highly significant correlation with pigmentation (table 4). The reason for this association is a bit puzzling, especially among northern Sudanese, for if it is due to linkage one might expect "light" to have higher frequencies of whorls than "darks" in view of the fact that southern Sudanese Negroes (Nilotes) have much lower whorl frequencies than do Middle Eastern peoples, or even northern Sudanese. There are two possible explanations for the association. First, perhaps Sudanese Negroes in the south western Sudan, whose dermatoglyphics have not been investigated, may possess a high frequency of whorls. They doubtless have constituted a sizable proportion of the Negro parent population. Second, there is a positive correlation between the occurrence of whorls on fingertips and patterns in the second interdigital area in various populations, suggesting one or more pleiotropic genes affecting

TABLE 3. ASSOCIATIONS BETWEEN ABILITY TO TASTE PHENYLTHIOCARBAMIDE AND PATTERNS IN SECOND INTERDIGITAL AREA

	PATTERNS		NO PATTERNS	
	Tasters	Non-tasters	Tasters	Non-tasters
100 Northern Sudanese	15	0	79	6
77 American Negroes	9	2	57	9
Total	24	2	136	15

$X^2 = 0.11$, df = 1, p between .95 and .70

TABLE 4. ASSOCIATIONS BETWEEN WHORLS AND PIGMENTATION

	DARK		LIGHT	
	Whorls	No whorls	Whorls	No whorls
34 American Negroes	70	120	35	115
100 Northern Sudanese	211	189	276	324
Total	281	309	284	466

$X^2 = 11.77$, df = 1, $p < .01$

both whorls on fingertips and patterns in the second interdigital area (Rife, 1943). We should expect whorls to be associated with the same traits as second interdigital patterns, if this be true. The association between whorls and patterns among American Negroes is in the direction one might expect, as many West African Negro populations are characterized by higher whorl frequencies than are North American Whites.

Unfortunately, the number of individuals tested for associations between blood groups and pigmentation among American Negroes is too small to be of any significance. An association is suggested, however, by the absence of any group B among "lights," and the similar percentages of A and B among "darks." It should be kept in mind that American Negroes are a relatively newer hybrid population than northern Sudanese, and that scarcely enough time has elapsed to eliminate all associations even between non-linked genes.

If linkage is indeed the reason for the association between second interdigital patterns and pigmentation, it suggests that two pairs of genes may be responsible for differences between Negroes and Whites, as proposed by Davenport (1913). If three or more independent pairs are concerned, it is difficult to see how linkage between only one pair with patterns could produce such an obvious association. There is some evidence that the presence of a pattern in the second interdigital area may be due to a simple dominant gene, lacking complete penetrance.

A collection of the palmar dermatoglyphics of 20 White American families assembled by the author reveals that among 15 families in which both parents lacked a pattern, only 3 out of 87 children possessed the pattern. All three occurred in the same family, in which 6 other children lacked the pattern.

TABLE 5. ABO BLOOD DISTRIBUTIONS

	%O	%A	%B	%AB
325 West African Negroes	52.30	21.50	23.00	3.20
20,000 Whites, U.S.A.	45.00	41.00	10.00	4.00
87 American Negroes	54.02	26.43	17.24	2.29
19 "dark" Negroes	52.63	21.05	26.31	0.00
16 "light" Negroes	56.25	42.75	00.00	0.00

Among 5 families in which one parent possesses the pattern, 11 out of 29 children manifest it. It occurs among the children in 3 of the families.

I wish to emphasize that the association between hand patterns and skin pigmentation suggests linkage, but does not necessarily prove it. Data from mixed Negro-White families should enable one to establish or reject linkage as the reason for the association. Linkage appears to be the most likely explanation, however.

Hybrid populations provide a virtually untapped reservoir of material which could be used to good advantage for the detection of linkage. They could be of special importance in tackling the problem as to whether or not ethnic groups differ in mental capacities, providing valid tests are used and emotional biases are not allowed to interfere with objective evaluations.

SUMMARY

1. Association between two genetic traits, and the absence of associations of either with a third genetic trait within hybrid populations, may under specified conditions be indicative of linkage.

2. Associations between hand patterns and skin pigmentation indicative of linkage were observed among northern Sudanese and American Negroes.

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A Further Note on Some Considerations of Heterozygote Detection

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IN A RECENT PAPER (Gartler, 1953) an investigation was made of a mating system based on the detection of the carriers of genes for mono-factorially determined recessive lethal traits. In this mating system $Aa \times Aa$ matings are prevented. It was shown that, for the case where the selective values of AA and Aa are equal, and where the resultant mutation pressure is from $A \rightarrow a$, the frequency of aa is reduced to ½ what it would be under natural selection. Furthermore, the frequency of Aa at the limiting point of this system is 0.5, which causes a severe restriction of mating combinations. Finally, some artificial selection has to be practiced at the limiting point of this system due to the excess Aa produced through mutation. One interesting case not covered in that paper is where the heterozygote has a selective advantage over both homozygotes. The purpose of this note is to analyze this case under two sets of conditions, mutation and no mutation.

Case 1. Selection in favor of Aa, no mutation, population size large and constant, migration absent, and mating monogamous.

In a population consisting only of AA and Aa, as will be the case here, where $AA + Aa = 1$, q, the frequency of a will be equal to ½ the proportion of Aa. Therefore, Aa will be equal to $2q$ and AA will be equal to $1 - 2q$. If we consider the selective value of Aa as unity, the selective value of AA will be $1 - S$, where S is the coefficient of selection against AA. Then, with the mating system under consideration, where $Aa \times Aa$ are prevented, aa genotypes will no longer appear. However, due to the higher selective value of Aa, the proportion of Aa in the population will increase up to the limiting point for this mating system ($Aa = AA = 0.5$; $q = 0.25$). The increase of Aa will take place at a more rapid rate than for the case where the selective values of AA and Aa are equal and mutation is the sole factor accounting for the recurrent appearance of aa.

The proportion of the two genotypes AA and Aa in the population, before and after selection, are shown below:

	AA	Aa	Frequency of q
Before Selection	$1 - 2q$	$2q$	q
After Selection	$(1 - 2q)(1 - S)$	$2q$	$\frac{q}{1 - S + 2qS}$

[1] This work was done while the author was the holder of a U. S. Public Health Service Postdoctoral Research Fellowship.

The general relation between two consecutive values of q is:

$$q_{n+1} = \frac{q_n}{(1 - S)(1 - 2q_n) + 2q_n}$$

its general term being:

$$q_n = \frac{q_0}{(1 - S)^n(1 - 2q_0) + 2q_0}$$

where q_0 is the initial frequency of a at the institution of this mating system and n is the number of generations of selection after its inception. For comparative purposes, I have calculated the number of generations required for q to reach its limiting point (0.25), under this mating system, for the cases (1) where mutation alone (selective values of AA and Aa equal) accounts for the recurrent appearance of aa, and (2) where selection in favor of Aa alone accounts for an assumed equilibrium condition. Two values of q have been used, 0.01, and 0.02, which correspond to estimates of gene frequencies for Tay-Sachs disease (Slome, 1933) and for Thalassemia (Neel, 1950) respectively. Both of these diseases are lethal recessives, and in the case of Thalassemia, the heterozygote has been detected. In table 1 are presented the results of these calculations and, as can be seen, the rate of increase up to the limiting point for the case of selection in favor of Aa is much more rapid than for that of mutation from $A \rightarrow a$.

After the limiting point is reached, there will continue to be an excess of heterozygotes (Aa) due to their higher selective value. Since this excess would not be permitted to mate, this would result in a relative decrease in population size at a rate of S per generation unless, of course, there were a proportional increase in the birth rate. It is of interest, in this connection, to note that if selection acts only after mating, and is not gametic (that is, $Aa \times AA$ matings are more fertile than $AA \times AA$ ones), then, once the limiting point of this system is reached ($q = 0.25$), the selective advantage of Aa will disappear. Or to put it another way, S, the selection coefficient against the AA genotype,

TABLE 1. NUMBER OF GENERATIONS REQUIRED FOR q TO REACH 0.25

MUTATION FROM A TO a ALONE			SELECTION IN FAVOR OF Aa	
q	u[1]	Number of Generations[2]	S[3]	Number of Generations
0.01	0.0001	3900	0.01	380
0.02	0.0004	950	0.02	160

1. $u = q^2$

2. $q_n = \frac{u}{3u - 2q_n u} - \left(\frac{u}{3u - 2q_n u} - q_0\right) e^{-n(3u-2q_n u)}$

3. $S = \frac{q}{p}$

is a function of the frequency of AA and becomes zero when $AA = Aa$. This, of course, is simply due to the fact that, at this point, all matings would be of the $AA \times Aa$ type, and the offspring produced by these matings will be ½ AA and ½ Aa.

Case 2. Selection in favor of Aa, mutation $A \rightarrow a$, no migration, population size large and constant, and mating monogamous.

Where both selection in favor of Aa and mutation from $A \rightarrow a$ account for an equilibrium condition of a recessive lethal, the results of this special mating system will be a combination of the cases for selection and mutation alone. The rate of increase of q will be approximately equal to the sum of the increases due to selection and mutation, the general relationship between two consecutive values of q being:

$$q_{n+1} = \frac{q_n}{(1 - S)(1 - 2q_n) + 2q_n} + u\left[1 - \frac{q_n}{(1 - S)(1 - 2q_n) + 2q_n}\right],$$

the net result being higher than for the case of mutation alone, but lower than for that of selection alone. The aa genotype will never be completely eliminated due to the recurrent mutation pressure $A \rightarrow a$, and at the limiting point of this system, the frequency of aa will be approximately ½ u. As in the last case, at the limiting point of this mating system there will be an excess of Aa, here due both to selection in favor of AA and to the recurrent mutation pressure $A \rightarrow a$. The excess will be approximately S + u per generation, which unless otherwise compensated for, would mean a corresponding decrease in population size per generation.

SUMMARY

The consequences of a mating system in which $Aa \times Aa$ matings are not permitted and aa is lethal have been examined under the condition of the heterozygous genotype (Aa) having a higher selective value than the homozygous normal genotype (AA). The general results are as follows: 1. The lethal genotype is effectively eliminated for the case where selection in favor of the heterozygote alone accounts for the maintenance of the lethal gene in the population. 2. The frequency of Aa increases to 0.5 which is the limiting point of this system. 3. At the limiting point of the system an excess of Aa continues to be produced because of their selective advantage and consequently have to be eliminated by artificial selection.

ACKNOWLEDGEMENT

The author would like to thank Dr. F. J. Kallmann for his reading of the manuscript and his helpful suggestions.

ERRATA

I would like to take this opportunity to correct a mathematical error in my last paper on this same subject (Gartler 1953). The error was in assuming that selection was not effective under this special mating system over the range of q considered (0.0–0.25). In other words, it was felt that the number of *aa*'s produced through mutation would be negligible, thereby making the selection pressure nil. However, as the frequency of the lethal gene, q, approaches the limiting value of this system, selection pressure becomes significant, in that more than an insignificant number of *aa*'s are produced through mutation. Consequently, the published values for the times in generations required for the lethal recessive gene to reach the limiting value of the mating system are low. The equations given below take account of the selection pressure omitted in the original equations, and give values which very closely approximate the true ones for the entire range considered.

For the case of mutation from $A \rightarrow a$ the following expression has been derived:

$$q_n = \frac{u}{3u - 2q_n u} - \frac{u}{3u - 2q_n u} - q_0 e^{-n(3u-2q_n u)}.$$

For this same case, Dr. Dempster, of the University of California, has suggested a somewhat simpler equation, both in terms of derivation and handling:

$$n = \frac{\ln (1 - q_0) - \ln (1 - q_n)}{u\sqrt{(1 - 2q_0)(1 - 2q_n)}}.$$

And finally, for the case of mutation both ways:

$$q_n = \frac{u}{3u + v - 2q_n u} - \left(\frac{u}{3u + v - 2q_n u}\right) e^{-n(3u+v-2q_n u)}.$$

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Linkage in Man. Pelger's Nuclear Anomaly, Taste, and Blood Groups[1]

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THE anomaly of the granulocytic leucocyte, consisting of lack of segmentation and condensation of the nucleus, was described by Pelger (1928). Huët (1932) disclosed the hereditary nature of the anomaly and subsequent investigators have confirmed the hematologic and genetic features of this disorder. During the course of a hematologic survey, a case of Pelger's anomaly in an adult Japanese female patient was discovered. Yamasowa et al. (1953) investigated individuals in four generations of this family and detected 25 additional cases. The genetic data obtained supported Huët's concept of the simple dominant inheritance of this disorder.

There are comparatively few reports in the literature on Pelger's anomaly and as far as can be determined, no linkage studies have been carried out on this disorder.

METHODS AND MATERIALS

The pedigree and hematologic data have already been published by Yamasowa et al. (1953). Subsequent studies have revealed two additional cases in this family, and a total of 104 individuals have been examined. The revised pedigree is shown in figure 1. Blood was typed for ABO, MN, Rh and Kell. Taste sensitivity was determined by the use of phenylthiocarbamide test paper. The blood groups, taste sensitivity, presence or absence of the nuclear anomaly and other details are shown in the Appendix.

RESULTS OF THE INVESTIGATION

There is clearly no evidence of partial sex linkage. The red cells in all the cases reacted to Anti-D and did not react to Anti-Kell. No tested parent was both MN and affected with Pelger's anomaly. Therefore, of the linkage testers used, only the ABO blood group, the *C*, *c*, and *E* reactions of the Rh locus, and PTC taste sensitivity are informative in this pedigree. Calculations of linkage scores are shown in tables 1 and 2, using the u statistics of Fisher (1935) as modified by Finney (1940).

Received October 29, 1953.

[1] This work was sponsored by the Atomic Bomb Casualty Commission, Field Agency in Japan of the Committee on Atomic Casualties, National Academy of Sciences—National Research Council, under a contract with the U. S. Atomic Energy Commission.

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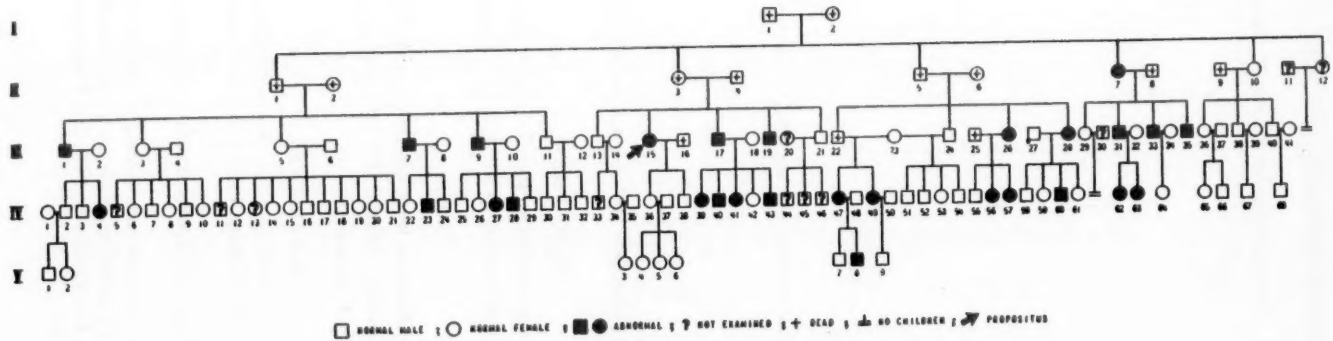

FIG. 1. Pedigree of Pelger-Huët Anomaly

TABLE 1. LINKAGE SCORES OF "CERTAIN" FAMILIES

TESTER	PARENTS	FINNEY TYPE	a	b	c	d	s	λ	k
ABO	III.1,2	1	—	1	2	—	3	3	3
	III.27,28	1	—	1	2	—	3	3	3
	III.31,32	1	1	1	—	—	2	−1	1
	IV.47,48	1	1	—	—	1	2	1	1
Rh	III.1,2	3	—	1	1	1	3	−1	3
	III.9,10	3	—	2	1	2	5	−2	10
	III.17,18	3	2	2	—	1	5	−2	10
PTC	III.1,2	2	—	1	2	—	3	.778	.550
	III.7,8	2	—	1	2	—	3	.778	.550
	III.9,10	1	2	—	1	2	5	2	10
	III.17,18	1	3	1	1	—	5	−2	10

Symbols refer to Finney (1940).

TABLE 2. LINKAGE SCORES OF "DOUBTFUL" FAMILIES (ABO)

PARENTS	FINNEY TYPE	a	b	c	d	s	λ	k
II.7,8	1a	2	1	1	—	4	−1.050	1.682
III.17,18	2	4	—	1	—	5	0.068	0.012

Japanese ABO gene frequencies taken from McArthur and Penrose, Ann. Eug. 15: 305 (1951).

CONCLUSIONS

This family reveals no indication of a close linkage of the genes for PTC taste sensitivity, ABO, or Rh blood groups to the gene for Pelger's anomaly.

NOTE

The sera used in this investigation were kindly provided by Dr. John Elliot of the Blood Bank of Dade County, Miami, Florida, and the PTC test paper by Dr. Chozo Oshima of Osaka University.

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APPENDIX

Key to Rh Reactions

CDe = C+ c− D+ E− CcDe = C+ c+ D+ E− cDe = C− c+ D+ E−
CDE = C+ c− D+ E+ CcDE = C+ c+ D+ E+ cDE = C− c+ D+ E+

No.	*Sex*	*Year of birth*	*Pelger*	*ABO*	*MN*	*Rh*	*PTC*
II.7	F	1885	+	A	M	CDe	−
II.10	F	1890	−	A	M	CDe	−
III.1	M	1897	+	A	M	CcDE	+
III.2	F	1903	−	B	N	cDE	+
III.3	F	1903	−	O	M	CcDe	−
III.4	M	1898	−	O	MN	CcDe	+
III.5	F	1908	−	A	M	CcDe	−
III.6	M	1902	−	AB	MN	CcDe	+
III.7	M	1913	+	O	M	CDe	+
III.8	F	1916	−	AB	N	CDe	+
III.9	M	1915	+	O	M	CcDE	+
III.10	F	1915	−	O	M	CcDe	−
III.11	M	1918	−	O	M	CDe	+
III.12	F	1922	−	O	M	CDe	+
III.13	M	1898	−	A	M	CDe	−
III.14	F	1900	−	AB	MN	CcDE	+
III.15	F	1903	+	O	M	CDe	−
III.17	M	1906	+	A	M	CcDe	+
III.18	F	1908	−	A	MN	CcDE	−
III.19	M	1907	+	O	M	CDe	+
III.23	F	1911	−	AB	M	CDe	+
III.24	M	1912	−	O	M	CDe	+
III.26	F	1923	+	O	M	CDe	+
III.27	M	1911	−	B	MN	CcDE	+
III.28	F	1919	+	A	M	CDe	+
III.29	F	1907	−	A	M	CDe	−
III.31	M	1920	+	A	M	CDe	−
III.32	F	1927	−	B	M	CDe	−
III.33	M	1924	+	A	M	CDe	−
III.34	F	1929	−	B	N	CDe	−
III.35	M	1928	+	O	M	CDe	+
III.36	F	1907	−	A	M	CcDE	+
III.37	M	1902	−	AB	MN	CcDE	+
III.38	M	1921	−	A	M	CDe	+
III.39	F	1926	−	O	MN	CcDE	+
III.40	M	1928	−	O	M	CDe	+
III.41	F	1930	−	A	MN	CDe	+
IV.1	F	1926	−	B	MN	cDe	+

APPENDIX—*Continued*

No.	*Sex*	*Year of birth*	*Pelger*	*ABO*	*MN*	*Rh*	*PTC*
IV.2	M	1922	–	AB	MN	cDE	+
IV.3	M	1927	–	AB	MN	CcDE	+
IV.4	F	1936	+	B	MN	CcDE	–
IV.6	F	1930	–	O	M	CcDe	–
IV.7	M	1932	–	O	M	CcDe	+
IV.8	F	1934	–	O	MN	CcDe	+
IV.9	M	1936	–	O	M	CcDe	+
IV.10	F	1939	–	O	MN	CcDe	+
IV.12	F	1930	–	AB	MN	CcDe	+
IV.14	F	1936	–	A	MN	CcDe	+
IV.15	F	1937	–	AB	MN	CcDe	+
IV.16	M	1939	–	B	M	CcDe	+
IV.17	M	1941	–	B	M	CDe	+
IV.18	M	1943	–	A	M	CcDe	+
IV.19	F	1945	–	A	M	CcDe	+
IV.22	F	1939	–	B	MN	CDe	+
IV.23	M	1944	+	A	MN	CDe	–
IV.24	M	1948	–	A	MN	CDe	+
IV.25	M	1936	–	O	M	CcDE	–
IV.26	F	1938	–	O	M	CcDE	–
IV.27	F	1941	+	O	M	CcDE	+
IV.28	M	1944	+	O	M	CcDE	+
IV.29	M	1948	–	O	M	CcDe	+
IV.30	M	1947	–	O	M	CDe	+
IV.31	M	1948	–	O	M	CDe	?
IV.32	M	1951	–	O	M	CDe	?
IV.34	F	1928	–	B	M	CcDE	+
IV.35	M	1925	–	A	M	CcDE	+
IV.36	F	1921	–	O	MN	CDe	–
IV.37	M	1919	–	O	M	CDe	+
IV.38	M	1934	–	B	MN	CcDe	–
IV.39	F	1930	+	A	MN	CcDe	–
IV.40	M	1934	+	A	M	CcDE	+
IV.41	F	1940	+	A	M	CcDe	+
IV.42	F	1944	–	A	M	CcDE	+
IV.43	M	1947	+	A	MN	CDe	+
IV.47	F	1927	+	B	M	CDe	+
IV.48	M	1920	–	A	M	CcDE	+
IV.49	F	1930	+	B	M	CDe	+
IV.50	M	1926	–	A	MN	CDe	+

APPENDIX—*Concluded*

No.	*Sex*	*Year of birth*	*Pelger*	*ABO*	*MN*	*Rh*	*PTC*
IV.51	M	1935	−	A	M	CDe	+
IV.52	M	1940	−	A	M	CDe	+
IV.53	F	1944	−	A	M	CDe	+
IV.54	M	1946	−	A	M	CcDe	+
IV.55	M	1942	−	B	MN	CDe	+
IV.56	F	1944	+	O	MN	CDe	+
IV.57	F	1946	+	O	MN	CDe	+
IV.58	M	1940	−	A	M	CDe	+
IV.59	F	1943	−	AB	M	CcDE	+
IV.60	M	1946	+	O	MN	CcDE	+
IV.62	F	1948	+	B	M	CDe	−
IV.63	F	1953	+	AB	M	CDe	?
IV.64	F	1952	−	AB	MN	CDe	?
IV.65	F	1932	−	AB	MN	CcDE	+
IV.66	M	1936	−	B	M	CcDE	+
IV.67	M	1949	−	A	M	CDe	+
IV.68	M	1950	−	A	MN	CDe	−
V.1	M	1948	−	B	N	cDE	−?
V.2	F	1951	−	A	N	cDE	−?
V.3	F	1951	−	A	M	CcDE	+?
V.4	F	1946	−	O	M	CDe	+
V.5	F	1948	−	O	M	CDe	+
V.6	F	1950	−	O	M	CDe	−?
V.7	M	1948	−	A	M	CDe	+
V.8	M	1950	+	B	M	CcDE	+
V.9	M	1951	−	A	MN	CDe	−?

Conference On Problems and Methods in Human Genetics

PROCEEDINGS

Edited by

WALTER E. HESTON

National Cancer Institute, Bethesda, Md.

Held Under the Auspices of

THE MORPHOLOGY AND GENETICS STUDY SECTION

Division of Research Grants

National Institutes of Health, Public Health Service

and

THE AMERICAN CANCER SOCIETY

upon recommendation of

The Committee on Growth, National Research Council

Kenwood Club

Bethesda, Maryland—October 8 and 9, 1953

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Present address: Children's Cancer Research Foundation, Boston

PROGRAM

October 8

Morning Session—SAMPLING TECHNIQUES AVAILABLE

Moderator: Dr. Curt Stern, University of California
Speakers: Dr. James V. Neel, University of Michigan
Dr. F. E. Stephens, University of Utah
Dr. C. Nash Herndon, Bowman Gray School of Medicine

Afternoon Session—SELECTION OF PROBANDS AND CONTROLS

Moderator: Dr. C. C. Little, Jackson Memorial Laboratory
Speaker: Dr. Madge T. Macklin, Ohio State University

Evening Session—PROBLEMS CONFRONTING HUMAN GENETICISTS—DISCUSSION

Moderator: Dr. Laurence H. Snyder, University of Oklahoma

October 9

Morning Session—ANALYSIS OF MECHANISMS OF INHERITANCE

Moderator: Dr. Earl L. Green, Atomic Energy Commission
Speakers: Dr. William J. Schull, University of Michigan
Dr. J. N. Spuhler, University of Michigan

Afternoon Session—TWIN DATA

Moderator: Dr. Frederick Henry Osborn, The Population Council, Inc.
Speaker: Dr. Franz J. Kallmann, New York State Psychiatric Institute

Evening Session—LOOKING TO THE FUTURE—DISCUSSION

Moderator: Dr. C. P. Oliver, University of Texas

Participants

Gordon Allen M.D., N. Y. State Psychiatric Institute
V. Elving Anderson, University of Minnesota
J. H. Bennett, Carnegie Corporation
William C. Boyd M.D., Boston University
W. G. Cochran, Johns Hopkins University
R. E. Comstock, North Carolina State College
Robert Cook, Journal of Heredity
Paul R. David, University of Oklahoma
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F. C. Fraser M.D., McGill University
B. Glass, Johns Hopkins University
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Earl Green, Atomic Energy Commission
B. G. Greenberg, University of North Carolina
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W. E. Heston, National Cancer Institute
Franz J. Kallman M.D., New York Psychiatric Institute
Tage Kemp M.D., University Institute of Human Genetics, Copenhagen
A. M. Lilienfeld M.D., Johns Hopkins University
C. C. Little, Roscoe B. Jackson Memorial Laboratory
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Madge T. Macklin M.D., Ohio State University
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Conference on Problems and Methods in Human Genetics

INTRODUCTION

It is a part of the stated policy of the Division of Research Grants of the National Institutes of Health to sponsor from time to time working conferences in fields that are rapidly developing and in which problems are being defined and methods are not yet thoroughly crystallyzed. Discussion of several applications for grants that have come to the Morphology and Genetics Study Section, in which Human Genetics has been involved, suggested the timeliness of this Conference. As our plans materialized we learned that the Committee on Growth of the National Research Council was considering a similar conference. They graciously agreed to combine forces with us and a committee under the chairmanship of Dr. Oliver with members of both groups was appointed. To them we owe the program before us and to the Secretary, Dr. Walter Heston, the planning and arrangement of the details of the meeting.

As a key note to our discussions I would go back to fundamentals. The philosophers of science have emphasized its objectivity and impersonality. Everyone who has sat through our study section meetings must agree that however true this is ideally, it is far remote from reality. Science is a human endeavor and as such is afflicted by the frailties of mankind. Our preconceived notions creep into our data and we find them mixed with emotional overtones which cannot be concealed by statistical treatment. This seems to become progressively more evident as we approach the sciences that have to do with man himself. Perhaps it is necessary, for a man must have some motivation to do the difficult and sometimes thankless work of the scientist.

The redeeming feature of the scientist is his essential honesty. He himself is aware of his own limitations and the limitations of his methods. Frankness in dealing with them will assure the success of such a conference as this.

J. WALTER WILSON

Problems in the Estimation of the Frequency of Uncommon Inherited Traits[1, 2]

JAMES V. NEEL

University of Michigan, Ann Arbor, Michigan

IT IS my understanding that the present meeting is very much a working conference, wherein we discuss not only more or less standard approaches to certain problems but also, quite frankly, some of the troublesome technical and statistical issues which arise in the course of genetic studies and for which there may be no complete solution at the present time. My particular assignment today is to describe and illustrate some of the various techniques which may be utilized in estimating the frequency of uncommon inherited traits. This is a problem in which we at Michigan have been greatly interested for the past several years, in connection with attempts to estimate the rate of mutation of a number of human genes.

The approaches employed in estimating the frequency of uncommon or rare inherited traits fall in general into two groups. The first type of approach consists in a precise enumeration of the frequency of the trait in question in a population selected at random with reference to that trait, and may be termed the "direct sample" approach. The second type of approach consists of an attempt to estimate, by a variety of means, the number of cases of a particular trait occurring in a particular population of known size, but without an actual census of the population in question. This may be termed the "indirect sample" approach. The direct approach is obviously the more precise since, assuming accuracy of diagnosis, the only error involved is the sampling error. Frequently, however, especially with reference to the rarer traits, a complete census is either impossible or impractical, and one must resort to indirect approaches to the estimate. Each of these indirect approaches carries with it one or more sources of error which are superimposed on the sampling error already present. In the following discussion, I shall confine my remarks to several examples of the "indirect sample" approach, since not only are we forced to use this approach more frequently than the other, but it also poses more complex problems.

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[1] It is a pleasure to acknowledge the financial support in these studies of the American Cancer Society through the Committee on Growth, and of the Division of Biology and Medicine of the U. S. Atomic Energy Commission.

[2] Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

EXAMPLES OF THE "INDIRECT SAMPLE" APPROACH TO ESTIMATING THE FREQUENCY OF AN UNCOMMON TRAIT

It should immediately be made clear that there is no general formula for this indirect approach, each trait constituting a separate and distinct problem. Whenever the trait is one of medical significance, medical records constitute an obvious point of departure. But there are many different kinds of medical records, and wide variations in the completeness of ascertainment of different diseases. The records of any one hospital must be carefully scrutinized for sources of bias. For instance, the case roster of most university hospitals is heavily loaded with the rare and bizarre, referred to the university hospital as a court of last appeal. Attempts to combine the records of a number of hospitals often run into problems of professional cooperation. Quite aside from the other merits of the action, the medical geneticist sees much in the establishment of a state "central medical registry" to recommend it—providing, of course, that he has access to the records.

I turn now to a consideration of three specific examples of frequency estimates based on the indirect approach.

1. *The frequency of retinoblastoma*

Retinoblastoma is a highly malignant form of ocular neoplasm which usually makes its appearance in the early years of life and is unusual in that the development of the tumor appears to be conditioned by the presence of a single gene which is about 90 per cent penetrant. The only known therapy consists in the removal or intensive radiation of the affected eye or eyes. Some years ago Dr. Harold F. Falls and myself undertook a study of the rate of mutation of the gene responsible for this condition (Neel and Falls, 1951; Falls and Neel, 1951). One prerequisite for deriving a mutation rate is of course the most accurate estimate of the frequency of the trait possible. Because the tumor of retinoblastoma is so accessible to examination and the early age of onset so characteristic, it seemed quite likely that each case occurring in the state of Michigan in recent years had sooner or later been accurately diagnosed, although there might also be some false diagnoses. But how to locate these cases? In the end, a method which combined nine different approaches was used. By means of these approaches, an attempt was made to locate every case occurring in the State of Michigan between 1938 and 1947. In brief, these approaches were as follows:

1. The records of the Heredity Clinic and the University Hospital of the University of Michigan were surveyed. This brought to light 28 proven cases. As will be seen, this is almost half of the total, and reflects the "referral" nature of the University Hospital's practice.

2. The files of the office shared by Dr. Bruce Fralick, Dr. Harold Falls, and

(prior to his death) Dr. Albert Barr, all members of the Department of Ophthalmology of the University of Michigan School of Medicine with a large referral type of practice, yielded 28 cases, overlapping to a considerable extent but not entirely with the cases mentioned under (1).

3. The Department of Pathology of the School of Medicine conducts a diagnostic tissue service for a number of hospitals scattered throughout the state, as well, of course, as for the University Hospital. Through the courtesy of Dr. Carl V. Weller, Chairman of the Department of Pathology, a list of the sources of all the specimens—24 in number—referred for diagnosis during the period under consideration was made available.

4. Letters were written to the 214 hospitals in the State of Michigan (other than the University Hospital) whose bed capacity exceeded 10 and which were not exclusively devoted to the treatment of alcoholism or mental disease. A total of 147 of these hospitals answered a query concerning the admission of patients with retinoblastoma between 1938 and 1947, a response rate of 69 per cent. A list of 32 presumptive cases was established in this manner.

5. Letters of inquiry were written to 218 physicians in the state thought to be devoting all or a large portion of their time to ophthalmology (exclusive of Drs. Fralick and Falls). A total of 105 of these physicians replied, while in 18 instances the letter was returned because the address was incorrect, and in 6 instances the letter was returned because the physician was deceased. Exclusive of the two latter categories, the response rate was 54 per cent. Information was obtained in this fashion about 11 presumptive cases.

6. The State Department of Vital Statistics codes all death certificates on IBM cards and very courteously made available to us a listing of all persons for whom, between 1943 and 1947, the primary or contributory cause of death was stated to be cancer of the eye. The IBM cards corresponding to earlier deaths had been destroyed, so that a listing was not available for 1938–1942. There were 43 such certificates, on 4 of which there was a definite diagnosis of retinoblastoma. In 23 cases there was uncertainty concerning the exact histological diagnosis, and letters were written to the attending physician or family to clarify this point. Ten of these were answered, with information concerning one more case. The total of cases located by this approach was thus 5.

7. The Armed Forces Institute of Pathology kindly examined its files for accessions of retinoblastoma originating in the state and submitted to the Institute for diagnosis between 1938 and 1947. A record of one case was obtained in this manner.

8. A visit to the Michigan State School for the Blind yielded the names of three children currently in attendance whose blindness was due to bilateral retinoblastoma.

9. A brief newspaper account of the study resulted in two alleged cases being brought to our attention.

Through these nine sources a preliminary roster of 66 cases of retinoblastoma, representing 65 families, was established. Most of the names on the list were derived from at least two of the sources listed above, and some from three or four. A trained field worker then attempted to establish personal contact with each family and to obtain preliminary family and medical histories. It was possible to trace all but one of these families. Once contact had been established, the propositus (if alive), his siblings, and his parents were interviewed personally by Dr. Falls and myself, the interviews being carried out either at the Heredity Clinic or in the home. At this interview, the previously obtained data were verified; a more detailed medical history was obtained; ophthalmological examinations were carried out on as many members of the immediate family as possible, and, where circumstances permitted, a brief physical examination was also performed. As a result of these studies, 13 families were eliminated from the tentative list of retinoblastoma families, leaving 52 families, with 53 cases, for 48 of which pathological confirmation of the diagnosis was available. Of the five cases which have not been confirmed by a pathologist's report, in two the diagnosis is at least partially substantiated by the subsequent death of the child; in one the diagnosis was agreed to by four ophthalmologists; in one the diagnosis was verified for the child's identical twin sister, and in only one may the diagnosis be regarded as doubtful.

Retinoblastoma is typical of a number of rare inherited traits which are usually correctly diagnosed, and sufficiently uncommon that it is feasible for one group of investigators to study all the cases in a given area once they have been located. The actual estimation of the frequency of the trait, once a suitable case roster has been established, is of course quite simple. In this particular instance, the average age at diagnosis was two years. On the average, then, there is a two-year lag between birth and detection, for which reason, in calculating frequency, one will be more nearly accurate if one uses not births for 1938–1947 but births for 1936–1945. The disease is known to have developed in 52 among 1,054,985 births, a frequency of 4.9×10^{-5}. (A pair of affected identical twins was counted as a single case.) It should be emphasized that this is a minimum figure. Thus, a certain number of children capable of developing the tumor must be assumed to have died before its appearance. Furthermore, it would be too much to expect, in a study of this type, that one had located all the cases of the disease occurring in the state during the study period. On the other hand, from the manner in which information concerning any given case tended to come from several independent sources, it is unlikely that any substantial number of cases has gone unreported.

2. *The frequency of multiple neurofibromatosis*

Multiple neurofibromatosis is a condition characterized by numerous patches of brownish pigmentation, termed café au lait spots, and multiple tumors in-

volving both the peripheral and central nervous system. In affected individuals, café au lait spots are usually present at birth, increasing in number during the first several decades, but the tumors which characterize the disease usually do not appear until the second or even later decades, with a gradual increase in numbers and size thereafter. The tumors are usually predominantly peripheral in their distribution, but may be predominantly central, or a mixture of central and peripheral tumors may be present. Affected individuals are often of subnormal mentality. The syndrome in many families has the distribution to be expected of a trait due to a single dominant gene. However, frequently affected individuals fail to give a positive family history—under one hypothesis the appearance of the disease in these individuals may be attributed to mutation.

The diagnosis of this disease presents certain problems. Drs. Crowe and Schull (1953), in a recent paper on the diagnostic importance of café au lait spots which is a beautiful illustration of the value of the genetic approach in clarifying clinical entities, have shown that approximately 10 per cent of normal persons have one or more café au lait spots exceeding 1.5 cms. in their broadest diameter. Furthermore, 10 per cent of patients with proven neurofibromatosis have no café au lait spots of this size. However, 68 per cent of persons with proven neurofibromatosis had six or more café au lait spots, whereas no normal individual was found with this number of spots. It is thus apparent that although the presence of more than six café au lait spots will serve to identify approximately 70 per cent of persons with the disease and will not include any "false positives", in some 30 per cent of affected persons there may either be no such spots, or a number within the range encountered in normal individuals.

The peripheral tumors of this disease have a typically violaceous hue and firm consistency, and when palpated between the thumb and forefinger may slip into the underlying tissue in a manner referred to as "buttonholing". Although there is no justification for confusing the advanced development of tumors which may be seen in this disease with any other clinical entity, in the earlier stages, when only a few scattered tumors are present, they are often mistaken for multiple lipomas or sebaceous cysts.

It seems a reasonable estimate that the experienced observer, with the free use of X-ray to detect the skeletal manifestations of the disease, could in a randomly selected population diagnose some 90 per cent of the cases of neurofibromatosis present, the level of accuracy being better among the older age groups. Amongst practitioners of medicine as a whole, however, the diagnostic level is considerably lower. Furthermore, unless certain complications develop, such as pressure effects from one of the tumors, or the malignant degeneration of a tumor, or a growth disturbance, affected persons may not seek medical attention.

For the past three years Drs. Frank Crowe, Franklin Martin, Jr., W. J.

Schull, and myself have had in progress a study of the genetics of this disease, with particular reference to the rate of mutation of the gene or genes responsible for the condition. A satisfactory estimate of the frequency of the trait is again basic to any attempt to evaluate the mutation rate of the gene or genes concerned. I am sure it is apparent how very different our problem was here than in the example of retinoblastoma. By no means all cases in the population at large are diagnosed. Even if all the cases in an area with clear geographic limits, such as the State of Michigan, were known, the trait is sufficiently common that it would scarcely be feasible to attempt to investigate each reported case. Accordingly, we have had to resort to several methods of approximation which I would like to present at this time. As will become apparent, these general methods can be applied to a wide variety of inherited diseases, although, again as will become apparent, their really successful application requires a somewhat larger material than we have been able to assemble.

One of the frequent consequences of multiple neurofibromatosis, for reasons not now clear, is mental defect. This may be severe, but usually is of a lesser grade (Borberg, 1951). In a survey of 6,780 inhabitants of three Michigan institutions for the mentally deficient and one institution for epileptics, all from the Lower Peninsula of the state, 16 proven and three possible cases of neurofibromatosis came to light. Because of the effects of the disease on mentality mentioned above, the direct use of these findings in any effort to establish the frequency of the condition would result in an overestimate. However, in a total of 100 Lower Peninsula families intensively studied because one or more individuals in the family sought medical attention and was found to have neurofibromatosis, there were 213 living individuals with the disease, of whom one was in one of the above mentioned institutions. If we regard our own study series as unselected with respect to mentality, an assumption that is probably not entirely correct, then each case in an institution corresponds to 212 cases not institutionalized, and the number of cases in the Lower Peninsula of the State of Michigan can be estimated as between 16×213 and 19×213, or 3408 to 4047. In 1950, the population of the Lower Peninsula was 6,069,568 individuals. The frequency of the disease may thus be estimated at between 1 in each 1500 to 1781 persons.

The errors involved in this estimate are obviously formidable. We have made a few preliminary efforts at their evaluation, efforts so rudimentary that I hesitate to mention them before a group containing such distinguished representatives of the field of biometrics. Rather, I would like to pose the problem of the most efficient method of estimating this error (or confidence interval) as one on which we would greatly appreciate help from the discussants of this paper.

A second method of estimating the frequency of this disease which we have pursued is as follows: Between 1941 and 1950 88 patients with neurofibromato-

sis were diagnosed at the University Hospital. These patients were referred from all over the state. For the purposes of this calculation we will make the simplifying assumption, known to be incorrect for the Detroit area, that cases are drawn from the various counties and cities in proportion to their population. Fifty-nine of these 88 patients were subjected to intensive genetic studies, in consequence of which 71 additional cases of neurofibromatosis were discovered. During that same period of time, 10 such patients were diagnosed at the two largest hospitals in Kalamazoo, Michigan (information courtesy of Dr. Don Marshall). There was one patient who appeared in both series. Assuming that the proportion of known cases in the Kalamazoo area also seen at University Hospital is typical of the situation throughout the state, the size of the pool on which the University Hospital drew between the years 1941 and 1950 may be placed at $10 \times 88 = 880$. This number, now, is the number of patients seeking medical attention in the state between 1941 and 1950 who were found to have neurofibromatosis. Since in our own experience, family studies on each such patient bring to light on the average approximately one additional affected individual who has not sought care, the total number of cases in the pool on which the two areas are drawing can be estimated to be 1760. The estimated size of this pool may be taken as an absolutely minimum estimate of the number of patients in the state, since in our own data we can show that whereas approximately half of the population of the state is concentrated in the greater Detroit area, only 25 per cent of the patients seeking attention at the University Hospital came from that area. This undoubtedly reflects the concentration of good medical facilities in Detroit. Furthermore, the figure of 1760 is minimal because this embraces only a ten-year-period, so that affected persons seeking medical care during or prior to 1940 or subsequent to 1950 are not included. It seems not unreasonable to postulate that this estimated pool of 1760 corresponds to at least twice as many affected persons in existence throughout the state in 1950.

Here again, the errors of estimate are appallingly large, and here again I would appreciate help from the biometricians in arriving at the confidence limits of this estimate. We find it interesting that the two independent estimates agree as well as they do. The only other estimate of the frequency known to us is that of Preiser and Davenport (1918), who, without describing in detail their reasoning, place the frequency at 1 in 2000.

It should be pointed out that both estimates hinge on the occurrence of one individual who is represented in two independent samples. Had there been two such individuals, our frequency estimate would have been halved, while if there had been no overlaps, we would be unable to make a precise estimate, although we could still establish a "confidence level" for the frequency. I might say in passing that in planning the observations basic to the estimates we had anticipated a somewhat larger overlap between the two samples.

In retrospect, there are apparent a number of weaknesses in the design of the observations leading to these estimates, weaknesses which could not be entirely anticipated in advance. Two important determinants in the design of the estimate were the personnel situation and the populations available for study. For instance, we discussed the possibility of surveying Selective Service inductees as being an approach to a randomly selected population, but the manhours involved were prohibitive. Perhaps the discussants can indicate to us some practical approach to the problem which we have overlooked. In passing, I should say that the type of stratified sampling which I suspect they will recommend is quite expensive, to the point where it will require considerable education to bring sources of funds to accept such expenditures.

3. *Multiple polyposis of the colon*

The final trait which I would like to discuss is multiple polyposis of the colon, a disease characterized by the occurrence of extremely numerous polyps throughout the large intestine. There is a marked tendency for some one or more of these polyps to undergo malignant degeneration at a relatively early age. One aspect of a study of this disease in which Dr. T. Edward Reed, Dr. H. Marvin Pollard, and myself are engaged is again an attempt to arrive at a rough approximation to the frequency of the disease. In this instance we have proceeded as follows: The average age at death from carcinoma of the colon of affected individuals in the series of 23 Michigan families under study is 38.9 ± 2.2 years. This underestimates the true average age at death for all individuals with the trait, since some older affected individuals still living will later develop carcinoma of the colon. However, for the purposes of this calculation, we will take 40 as the average age at which individuals with multiple polyposis die. The Bureau of Vital Statistics of the State of Michigan has kindly supplied us with photostats of the death certificates of all persons dying at age 39 or below of carcinoma of the colon during 1950–1952 inclusively. This amounts to 103 certificates. Among these, there were two certain, and one possible, cases of multiple polyposis. Follow-up studies are in progress on the 100 individuals not stated to have polyposis; Dr. Reed has thus far obtained additional information concerning 57 of the families of these 100 deceased individuals without uncovering presumptive evidence for any family that multiple polyposis was an unrecognized predisposing lesion to the development of the carcinoma. For the three-year-period, then, there is an average of one case a year of death below the age of 40 due to carcinoma of the colon superimposed on multiple polyposis, from which it may be deduced that there are on the average approximately two deaths per year at all ages in the state because of the complications of multiple polyposis. The number of deaths in the State of Michigan each year is approximately 58,000. Multiple polyposis may thus be estimated to be present in

approximately one in each 29,000 persons dying in the State of Michigan. Because some persons with multiple polyposis die of unrelated causes, and because in some instances of inoperable carcinoma of the colon the studies necessary to establish multiple polyposis as the predisposing cause may not be carried out, this is a minimum estimate of the frequency of the condition. Again permit me to call your attention to the critical role played in this estimate by a relatively few cases.

The two frequency estimates we have derived suggest that neurofibromatosis is approximately 15 times as common as multiple polyposis of the colon. There is available a very simple but also very approximate check on the accuracy of that frequency ratio. This involves a comparison of the frequency of hospital admissions for the two diseases during some stated period. As noted above, between 1941 and 1950 88 patients were found to have multiple neurofibromatosis at the University of Michigan hospital. During that same period of time there were 11 diagnoses of multiple polyposis of the colon. This is approximately half the ratio of the frequency of the two diseases suggested above, but when one considers the magnitude of the errors involved in both estimates, as well as the fact that for medical reasons patients with multiple polyposis are more apt to see a physician than those with neurofibromatosis, the two sets of figures seem to be in satisfactory agreement.

CONCLUDING REMARKS

In the foregoing presentation I have deliberately selected examples emphasizing some of the problems which have arisen and the pitfalls in which we have come to grief in our efforts to determine the frequency of certain uncommon inherited traits. I would not like to leave the impression that we are alone in these troubles. A critical review of the frequency estimates which are basic to calculations concerning the rates of mutation of the genes responsible for hemophilia, aniridia, epiloia, Pelger's nuclear anomaly, or a number of other traits, reveals equal or even more glaring weaknesses. These problems are common to all of us interested in this field.

I am sure we will all agree that a complete census of a given population is the best way to determine the frequency of a given trait. Unfortunately, the cost of such a census is often prohibitive, there may be enough non-cooperation to introduce serious bias, and, in the case of medical traits, it is difficult for understandable reasons to find a physician willing to examine 20 or 30 thousand non-affected individuals for the sake of finding a handful with the trait under study. We are thus more or less forced to various approximation techniques. It is my feeling that neither the biological nor the statistical pitfalls in these techniques have been sufficiently emphasized in the past. In this presentation I have attempted to do no more than 1) sketch out several methods which we

have been exploring, 2) indicate certain shortcomings of these methods in our hands, and 3) lay out the statistical problems in such a way that our biometrical colleagues will be given full scope for their talents.

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Sampling Techniques Available in Human Genetics[1]

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AT THE University of Utah we have used extensively the study of family pedigrees in our work in human genetics. The state is well suited for this type of study. The families are large, and in many communities people tend to be more or less permanently located. The Mormon people are intensely interested in genealogy and in obtaining family histories. Copies of these are sent to the Church's Genealogical Library in Salt Lake City, where approximately two and a half million family group records are on file. In addition to this, the Church has a very extensive program of microfilming genealogical records of all kinds in foreign countries and in the United States. Many Mormon people trace back to polygamous ancestors thus providing unusually large family pedigrees.

Our general procedure is to interview as many members of the different kindreds as possible. We secure data regarding them and their relatives who are either inaccessible or dead. Arrangements are made for such examinations and tests as are deemed necessary.

It is important to be perfectly frank and honest with the families involved. The family must have assurance that their names will not be used in publications and that all unnecessary publicity will be avoided. Assurance is also given

[1] Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

that the study will not cost them any money and that any good that comes as a result of the study will be of benefit to them and their children. Where photographs are taken signed permission for their use is secured.

In our U.S. Public Health Service project on the study of muscular dystrophy and other hereditary and degenerative diseases, a cooperative study has been organized. We have a clinician who is equipped with a laboratory and a special ward at the hospital. He diagnoses cases, runs necessary tests and studies patients under controlled hospital conditions. A chemical division has a laboratory where chemical studies directly or indirectly connected with the project are made. The third division makes genetic studies, gathers family data, cooperates in arranging for the examinations, etc.

Whenever possible, individuals are examined in the hospital. If this cannot be done, arrangements are made for examinations to be given in the homes or at some accessible place. This is especially necessary where members of the kindred live in outlying communities. By examining a large sample of a large kindred we are able to see a trait in all of its forms of expression. The clinician is able thereby to detect many cases which would otherwise be missed and to eliminate other cases similar in appearance which might be mistaken for the trait being studied. Classifications based on the examination of only one or two individuals lead to confusion. In facioscapulohumeral muscular dystrophy, the expression of the same dominant gene in two individuals of the same kindred may be so different that it would not be recognized as the same trait were it not for its general pattern of inheritance.

Our sampling techniques can be best demonstrated by reference to some projects which we have undertaken. The first was a study of achondroplasia in a large Utah kindred. Information secured from family members and verified by other members was very valuable in this study since the trait can be easily recognized at an early age. The kindred is large enough to give a significant ratio of affected to normal individuals. By checking carefully on all branches of the kindred it was possible to secure good evidence that the trait had its beginning as a dominant mutation.

In a kindred showing facioscapulohumeral muscular dystrophy, there are 1259 people including over 150 affected individuals. The numbers are sufficiently large to indicate the significance of the genetic ratio secured. We have several large unrelated kindreds of this kind showing the same trait with the same pattern of inheritance. The question arises whether all should be studied in detail and reported. Childhood muscular dystrophy and facioscapulohumeral muscular dystrophy are often described as progressive muscular dystrophy. This is very confusing since facioscapulohumeral muscular dystrophy is inherited as a simple dominant while the childhood type is inherited as a sex-linked recessive.

Ten affected individuals was the largest number found in any one kindred of

the childhood type. Many kindreds had only one affected individual. Thirty-three different kindreds were studied and reported. Some of these showed good evidence of sex-linked recessive inheritance, others had too few individuals in the pedigree to indicate any type of inheritance, while still others having only one affected individual had sufficient unaffected males to raise a question about the trait being a sex-linked recessive. Our data were considered evidence that we were dealing with a gene that mutates at a comparatively high frequency. It was very essential in this study of kindreds showing muscular dystrophy to secure accurate data on the families not showing the trait.

Paramyotonia is another interesting muscular disease. In this disease, muscles show an abnormal sensitivity to cold. This trait occurs very early in life and is readily detected by the family. Mothers can detect the trait in babies soon after birth. Information secured from the kindred members is very valuable, and I think reliable, even with few examinations by a physician. This is quite in contrast to myotonia dystrophica which is a syndrome that varies greatly in the manner of its expression. Sometimes the only expression of the trait is a form of cataract. Interviews with relatives are extremely unreliable. Actual examinations by a competent physician are imperative for this study. The personalities of these people are often affected by the trait making cooperation difficult. For some reason they are often very loth to admit that they have any symptoms of the disease.

An unusual case of pyelonephritis found in a large kindred requires a laboratory test for diagnosis and is an example of a case where cooperative study including laboratory facilities is a necessity. In one community a temporary laboratory was set up and ninety-four related individuals were tested in two or three days. The disease does not give any unusual difficulty until early adult life. The affected male adults never recover, but gradually get worse until they die from uremic poisoning. While members of this kindred were carefully checked and whenever possible rechecked, we feel that the whole study should be re-done at a later date to make sure that the techniques used were adequate. The children showing positive tests were not ill so far as could be determined by an ordinary examination. When the adult males, however, gave a positive test they were ill. Twelve of them died from uremic poisoning. No females have suffered any serious effects although in many cases they gave a positive test.

In a study of hereditary nerve deafness we also went into the homes and took family histories. In addition, a physical examination and an audiometer reading were made by a laryngologist. A sufficient number of this kindred were studied to reveal a definite pattern of dominant inheritance.

We have found the genetics of human cancer a difficult problem to study by the family pedigree method. A trait which occurs in the general population at as high a rate as does cancer would be expected by chance alone to occur in high frequencies in certain kindreds. We have found high concentrations of

cancer in certain families but only in a few does a simple genetic pattern appear. Polyposis, a precancerous condition of the lower bowel, is one of these traits. Three kindreds showing this condition have been studied in our Laboratory. One of these containing more than 650 individuals has been studied in detail. In one branch of the kindred, sixty-nine descendants of one couple have been carefully studied and most of their living descendants subjected to a clinical investigation. The family histories were obtained through personal interviews. As a part of each interview, places of residence and names of doctors and hospitals were carefully noted. These leads were followed up as completely as possible. Death certificates for other people were reviewed. The leads obtained from this source concerning pathological findings from operations and autopsy reports were traced to their sources and original records were examined wherever possible.

A clinic for the examination of living members of the group was established. The routine examinations included a sigmoidoscopy, hematocrit, and guaiac test. Individuals showing positive results for either the sigmoidoscopy or guaiac test, and those having a history of digestive disturbance and bleeding from the rectum, were examined further by means of air contrast barium enema.

In a few cases where the distance was too great to bring the people to the hospital, the cooperation of private physicians was obtained to make the examinations. The data for these, including x-ray films, were obtained from their doctors. Dr. Eldon J. Gardner, who worked on this problem, has shown that a simple dominant gene is responsible for polyposis. The study of this kindred also emphasizes the importance of making careful general examinations of the patients. The first examination concerned itself only with polyposis. It did not note the presence of bony and soft tumors on the patients. Upon the request of Dr. Gardner a reexamination was made which showed a definite association of these tumors with the polyposis.

We are continuing to study breast and stomach cancer by the pedigree method. Where high concentrations of affected cases occur we are checking carefully the on-coming generation approaching cancer age to determine whether or not the trait is being transmitted. We should have a pretty good picture of how these types of cancer occur in kindreds even though the exact genetic pattern may not be evident.

An example of a kindred showing a high concentration of breast cancer is our Kindred 107. Whether or not this is a chance concentration we do not know. The farther back one goes from the present generation the less reliable is the information. We are keeping a careful check on the rising generation who are approaching, or are of, cancer age in this kindred to see if the trait is transmitted and to determine if possible any pattern of inheritance which might be suggested. The extent to which these family pedigree studies should be carried on is a problem.

We are not depending entirely on this type of study. Dr. Charles Woolf is investigating the genetics of human cancer by the statistical approach. So far he has been principally concerned with breast and stomach cancer.

Probands were selected from individuals who died from stomach cancer in the state of Utah during the years 1940 to 1950. Special care was taken to select only those individuals with good evidence of cancer. In many cases autopsy and operative reports were listed on the certificates. Upon obtaining the names of the probands and dates of death, obituary columns in the local newspapers were searched in order to procure the names of living relatives. Two hundred and twenty-two families were selected for study. A responsible member of each family was then contacted by mail or by personal interview.

The investigation of the families was confined to each proband's brothers, sisters and parents. Names were obtained along with the causes, places, and dates of death of those who were deceased. Information as to the occurrence of malignant or benign growths was noted as well as hospitals attended or operations undertaken for such neoplasms. As far as possible death certificates of all relatives who died were examined and cause of death noted. The study was continued until the goal of 200 family histories was reached.

Appropriate comparisons were made with Utah vital statistics. Conclusions showed no increased incidence of general cancer deaths in families of probands as compared with that in the general population, but, the number of deaths due to stomach cancer was significantly higher. This indicated an organ-specific predisposition.

Since this difference might have been due to some environmental condition such as diet, etc., a study was run on 540 spouses of individuals who had died from gastric cancer. These spouses showed no higher cancer frequency than that which was found in the general population.

We feel that the sampling technique to be used in any study depends upon first, the nature of the trait in question and second, upon methods which are available. A combination of all techniques available is often essential to an understanding of the problem involved.

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Three North Carolina Surveys[1]

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With respect to disease processes in which genetic factors are known or thought to be of etiologic significance, a major objective of population sampling

[1]Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

techniques is to obtain a series of families for study that will be as representative as possible of the base population. Certain population studies with this objective have been carried out by the Department of Medical Genetics of the Bowman Gray School of Medicine within the past fifteen years, and three of these will be reviewed with regard to evaluation of the effectiveness of the sampling technique.

MUSCULAR DYSTROPHY SURVEY

A survey of cases of progressive muscular dystrophy was begun in 1939 under the direction of Dr. William Allan in association with Dr. Paul David, and continued by the staff after Dr. Allan's death in 1943. An attempt was made to study all patients with this disease that could be discovered in the North Carolina population from 1921 through 1940. Original case records were obtained from the twenty-two orthopedic clinics then operated at various places throughout the state under auspices of the Crippled Children's Division of the State Board of Health, records of the North Carolina Orthopedic Hospital in Gastonia, Duke University Hospital in Durham, and from the private records of the orthopedic surgeons staffing these various clinics. These sources were chosen because of the clinical observation that practically all young individuals with chronic disabling neuromuscular disease are eventually referred to an orthopedist for examination. The experience of all Board certified orthopedic surgeons in North Carolina would be included in the original record group.

All original records were reviewed by a physician. Cases were accepted for inclusion in the survey group if they met any one of the following five criteria:

1. Diagnosis of muscular dystrophy of any type.
2. Diagnosis of peroneal atrophy of any type.
3. Diagnosis of muscular atrophy unless this was clearly secondary to a nonpertinent primary disease.
4. Diagnosis of neuromuscular disease without further classification.
5. All records undiagnosed or with questioned diagnoses if the symptoms or signs described suggested the possibility of muscular dystrophy.

By this method 278 case records were accepted for survey. It was found that certain sources of error and possible bias existed in the original records consulted. These included a lack of uniformity in diagnostic terms and diagnostic criteria. For example, diagnostic terms such as "progressive muscular atrophy" were encountered, it being found that one physician might be using this term to describe cases of muscular dystrophy while another physician might be using the same term to describe cases of progressive spinal muscular atrophy. The possibility of diagnostic error was always present, and it was found that patients with peroneal atrophy (Charcot-Marie-Tooth syndrome), particularly of the recessive variety, were frequently confused with cases of progressive

muscular dystrophy, and for this reason all cases diagnosed as peroneal atrophy were included in the survey group. An age limit existed on admissions to the twenty-two state orthopedic clinics and the North Carolina Orthopedic Hospital of 16 years, which resulted in a strong bias in favor of patients with age of onset under age 16. As we were primarily interested in the childhood variety of muscular dystrophy, this source of bias was considered unimportant for this particular study. The clinic records would also provide an over-representation of patients of limited economic status, but an effort was made to minimize the economic bias by inclusion of private records of the physicians staffing the charity clinics.

The list of 278 index cases selected for survey was thought to contain all detectable cases of progressive muscular dystrophy plus a group of other conditions which might be clinically confused with it. Each record was assigned an index number and a special file folder to contain all available records. On a large wall map of North Carolina pins and tags were inserted marking the last known address of the patient, and with different colored tags and pins used to indicate the patient's diagnosis and his status regarding completeness of data. Each index case that could be located was visited by a team consisting of a physician and a field worker, or at times by a physician alone. Physical examinations were done on all surviving patients and all relatives with any suspicious abnormalities, and medical data were collected for all patients from family physicians, hospital records or other available sources. Certain minimum pedigree data were obtained on all patients, including information concerning the sibship of the patient, both parents and any other direct ancestors on whom information was readily available. Where a clinical diagnosis of progressive muscular dystrophy was confirmed or suspected, the pedigrees were extended to include all relatives and ancestors known to the immediate family of the patient, and further visits were made to the homes of more distant relatives to confirm and extend the data. Inquiry was particularly made for any relatives with any type of crippling disease, and any patients so reported were followed wherever possible until a definite diagnosis of the type of abnormality could be determined. As the original diagnoses of the index cases were either confirmed or disproved by examination, the map tag colors were changed so that a current visual record was always available of the status and location of all cases. Patients that were in any way doubtful clinically were followed and reexamined or referred to hospitals or clinics for further study until satisfactory diagnoses were established for all index and secondary cases.

The survey results may be summarized in the two following tables, (tables 1, 2) the first listing the disposition of each of the 278 index cases and the second listing all diagnoses other than progressive muscular dystrophy and peroneal atrophy that were established.

Although this sample was designed to locate all cases of this disease that

TABLE 1. THE DISPOSITION OF 278 NORTH CAROLINA INDEX CASES

	INDEX CASES	KINDREDS	SECONDARY LIVING	CASES DEAD
P.M.D.	133	101	6	34
Peroneal Atrophy	61	51	126	46
Other Diagnoses	54	42	13	3
Diagnosis Uncertain	4	4	—	—
Outside N. C.	8	8	?	?
Unable to Locate	15	15	?	?
No disease	2	2	—	—
Duplicated Name	1	0	—	—
Totals	278	223	145	83

TABLE 2. DIAGNOSIS OF 278 NORTH CAROLINA INDEX CASES

DIAGNOSIS	INDEX CASES	KINDREDS	SECONDARY CASES
Cerebral Degeneration	18	7	5
Prog. Spinal Muscular Atrophy	11	11	—
Poliomyelitis	7	7	—
Traumatic Peroneal Atrophy	5	5	—
Amyotrophic Lateral Sclerosis	2	2	3
Malformations	2	2	—
Periodic Paralysis	2	2	1
Spinal Cord Lesions	2	2	—
Calcinosis	1	1	—
Friedreich's Ataxia	2	1	1
Microcephaly	1	1	2
Spastic Paraplegia	1	1	4
Totals	54	42	16

could be discovered in a population of approximately 3,500,000, the case finding technique cannot be termed exhaustive. We have no reliable estimate of the efficiency of case finding. If an ascertainment rate is estimated using a maximum-likelihood method developed by Dr. Paul David, a value of .820 $\pm$ .057 is obtained. We feel that this is more likely to be an over-estimate than an under-estimate of the true rate. A second point noted is that there may be a racial difference in ascertainment rates, with the likelihood of ascertaining a white patient probably being higher than that for a non-white patient. The crude population case rate for white cases was 5.37 per 100,000, while the corresponding rate for non-white cases was 3.49 per 100,000, the difference between these figures being slightly more than 2.5 times its standard error (1.88 $\pm$ 0.746). This difference may be due to a real gene frequency difference in the two racial groups, but could equally well represent a difference in case finding efficiency.

In overall evaluation of the method used, we find that its major disadvan-

tages, in addition to the possible sources of error and bias previously mentioned, are that it is quite time consuming, requires a great deal of travel and is quite expensive. However, the method offers certain advantages which we feel are important. First, personal examination of each patient and relative by one or both of the physicians responsible for the clinical material results in the uniform application of diagnostic standards and increases considerably diagnostic accuracy. We feel that the procedure of visiting a number of informants in each kindred for confirmation of and extension of pedigree data increases the accuracy of the pedigree data considerably over anything that could be obtained through interviews made in a hospital or clinic. We feel that the extensive investigation possible on each family does much to justify the large expenditure of time and travel involved in data collection.

SURVEY OF HEREDITARY BLINDNESS

A survey was undertaken in 1941 with the objective of estimating the proportion of blindness due to morbid inheritance in the North Carolina population and to obtain some estimate of the relative frequencies of various types of hereditary blindness. As we were primarily interested in blindness affecting children and young adults, only those conditions with onset earlier than age 60 years were considered.

The North Carolina State Commission for the Blind is required to maintain a register of all citizens known to have total vision less than 20/100 with correction. The law requires that physicians report any patient with vision of less than this level to the Commission. Our investigation was based on patients included in this register, but only a limited geographic area consisting of ten counties was covered. This area was subdivided into two portions with certain population characteristics. Guilford County, which is the largest county in North Carolina in population, was chosen as an urban and industrialized area, the entire county being classed as a "metropolitan area" by the United States Bureau of Census. The second subdivision consisted of nine small counties in the mountains having a combined population somewhat less than that of Guilford County, these counties being predominantly rural with very little industry of any kind. A total of 544 individuals were registered as blind in the urban and rural areas thus established. The numbers and registration rates for these areas are as follows.

	Urban	*Rural*	*Total*
Population, 1940	153,916	124,482	278,398
Total registered blind	242	302	544
Rate, registered per 1,000	1.57[1]	2.43[1]	1.95

[1] Difference in rates = 0.86 ± 0.172.

It is noted immediately that the registration of individuals as blind is significantly different in the urban and rural areas chosen, the difference in registra-

tion rates being five times its standard error. Although it is possible that significant differences may exist in the overall rates of blindness in these two areas, it seems even more likely that the observed difference might reflect a difference in completeness of registration. With regard to registries of this sort and with respect to any reportable disease, the question of the degree of under-registration becomes quite important. The state agencies in North Carolina do not provide any official estimate of the degree of under-registration represented in the records of the State Commission for the Blind. It seems obvious that in North Carolina the reporting of blindness is far less effective than reporting of infectious diseases and other vital statistics. It seems apparent that there is a large economic bias in the registration, with registration most complete for those of limited financial means, while registration is much less complete in the upper income brackets. This is influenced by the fact that financial aid, medical assistance and other services are made available through the Commission to those unable to afford such services through their own resources.

Of the total group of registered blind in the areas chosen for survey, certain records were discarded without further investigation. Records were discarded that met any of the following criteria:

1. Examination by a board certified ophthalmologist with a conclusive diagnosis of blindness of environmental origin.
2. Diagnosis by an ophthalmologist of senile cataract.
3. Any patient with age of onset of visual difficulty later than age 60 years.

296 cases were not investigated further on this basis. Investigation of the remaining 248 names was carried out by home visit, with their disposition and classification being listed in the following table (table 3).

By combining all cases personally studied and those classified from the original records, we arrive at the following broad classification into hereditary, environmental and unclassified types of blindness (table 4).

The results of this study were rather disappointing and of doubtful validity.

TABLE 3. CLASSIFICATION OF 544 CASES OF BLINDNESS

Discarded from original record		296
Investigated by visit		248
Examined, hereditary	93	
Examined, environmental	116	
Examined, unclassified	9	
Unable to locate	26	
Deceased	2	
Critically ill	1	
Cooperation refused	1	
Totals	248	544

TABLE 4. CLASSIFICATION OF URBAN AND RURAL CASES OF BLINDNESS

	URBAN		RURAL		TOTAL	
	No.	%	No.	%	No.	%
Hereditary	34	14.0	59	19.5	93	17.1
Environmental	194	80.2	218	72.2	412	75.7
Unclassified	14	5.8	25	8.3	39	7.2
Totals	242	100.0	302	100.0	544	100.0

In extenuation, it should be mentioned that this study was a war casualty and was discontinued before the original plan of investigation could be carried out. The original plan called for a survey of thirty counties, rather than the ten accomplished, and for adding to the survey group names obtained from the private practice records of ophthalmologists in the area. We feel that this study did not obtain a representative sample for several reasons. First, the original register records contained a large economic bias. The inclusion of records on private patients would have reduced this bias, but might not have eliminated it entirely. Second, it was demonstrated that the registration rates were unequal for the urban and rural areas, which might be accounted for in part by differences in economic level, but the possibility of other sources of systematic error should be considered. It is apparent that we have no reliable estimate of the degree of under-registration involved in this population. In any event the sample size is too small for reliable gene frequency estimates of any of the specific diseases considered.

POLIOMYELITIS TWIN STUDY

A twin-family study was undertaken in 1949 to investigate the possibility of genetic influence on susceptibility to paralysis in poliomyelitis, this being aided by a grant from the National Foundation for Infantile Paralysis. The sampling objective was to obtain a series of families each containing a pair of twins, one or both twins having had paralytic poliomyelitis while the twin partner was living, and in the same household. The results of this study have previously been published (Herndon & Jennings, 1951). It was felt that hospital case records would not be an adequate source for original sampling, as previous experience with such records had convinced us that a patient was much more likely to be recorded as being a twin if the co-twin were also ill, and also if the twins were monozygous. It was felt that hospital records would give an underrepresentation of dizygous twins where only one twin was affected. The primary source of records selected was the infectious disease report card which the physician is required by law to file with the State Board of Health whenever a diagnosis of poliomyelitis is made. Through the kind cooperation of the Division of Epidemiology of the State Board of Health, photostatic copies were obtained

of all report cards filed during the years 1940 through 1948, totaling 4,213 cases. An attempt was then made to classify each individual in this group as being of single or multiple birth. The birth certificates of a sample of the original group were consulted, approximately 10% being classified by this means. It was found that this procedure was too slow and time consuming to permit such classification for all of the 4,213 cases. As each reported case carried the name of the reporting physician, a questionnaire was prepared and sent to the reporting physician requesting that he specify whether the patient was a twin, not a twin, or unknown. In the covering letter it was emphasized that patients should be classed as unknown unless the physician possessed definite knowledge of the case. Replies were received from 786 physicians. A further attempt was made to classify cases still remaining unknown by requesting information from the appropriate county health department. Patients that were included in the 10% sample for whom birth certificates were consulted were also included in the questionnaire group, and no instance of difference in classification from the two sources was recorded. By this method, 3,890 of the original cases (92.3%) were classified with regard to multiple birth, and 59 families were reported to contain twin pairs.

Home visits were then made to the 59 index families, details of data collection procedure being given in the previous report. Thirteen families were rejected after investigation as not meeting the requirements for inclusion in the final study, these being subdivided as follows.

1. Twin partner deceased	5
2. Moved out of N. C.	2
3. Propositus not twin, had twin sibs	2
4. Duplicate, different names	1
5. Diagnosis not acceptable	
Probable non-paralytic poliomyelitis	2
Other diagnosis established	1
Total rejected	13

In any twin study, the incidence of twins ascertained in the base population and the distribution of types of twins so determined can serve as an internal check upon the adequacy of the sample. The figures found for this study are as follows.

Distribution of twins:

Total cases classified	3890
Twin pairs found	55
Incidence: (1:70.71)	$1.414 \pm 0.189\%$
Control (Strandskov) (1:86.13)	1.161
Difference	0.253 ± 0.190

Ratio of twin types:

Ratio monozygous:dizygous	—observed	14:31
	—expected	15.06:29.94
	—X^2	0.12
Ratio like-sex:unlike sex (dizygous only)	—observed	12:19
	—expected	15.5:15.5
	—X^2	1.58

The above figures indicate that the total incidence of twins among the total cases classified is in agreement with the incidence for the total United States population as given by Strandskov (1945). Also, the ratio of monozygous to dizygous pairs observed agrees well with expectancy and also the ratio of like sex to unlike sex pairs among the dizygous twins. One set of triplets was encountered in this study which is not included in the above figures applying to twins only. This set of triplets consisted of an affected male and two non-affected females, the females being derived from a single ovum. This set is classed as two pairs of dizygous discordant twins in the following concordance table (table 5).

In evaluation of the sampling technique in this study, we are aware of no serious sources of bias. We feel that the registration of poliomyelitis cases was practically complete for the entire North Carolina population during the period covered. The Division of Epidemiology of the State Board of Health feels that under-registration with regard to diagnosed paralytic poliomyelitis is negligible. Examination of the sample showed no detectable evidence of economic, geographic or racial bias. Internal evidence indicates that the ratio of twin types shows a random distribution. Clinically acceptable diagnostic data were available for all patients. No secondary cases were encountered in the course of study which met criteria for inclusion in the original series. All secondary cases of poliomyelitis found among relatives of the index twins that occurred during the period 1940 to 1948 were found to have report cards in the original series. We therefore suggest that the group of families studied forms an acceptable sample of the base population.

In conclusion, it is hoped that it will not be out of place to offer certain recommendations regarding the publication of reports of genetic studies which

TABLE 5. CONCORDANCE OF TWINS WITH POLIOMYELITIS

	CONCORDANT	DISCORDANT	TOTALS
Monozygous	5	9	14
Dizygous	2	31	33
Totals	7	40	47

$X^2 = 6.81$, D. F. = 1.

seem to be of importance in the light of our own studies and from perusal of the literature.

1. It seems important that details of case finding techniques should be given in any published report. It would seem useful to know whether even a small series of cases represents the writer's total experience with the condition in question, a complete series observed under specified conditions, or selected cases reported because of unusual features.
2. It seems important to record in some detail the diagnostic data available for secondary cases. There is obviously a vast difference between a hearsay diagnosis reported by a relative regarding someone not available for examination, a clinical diagnosis reported by a family physician, and a diagnosis confirmed by careful autopsy. Many reports in the literature provide no basis for assessing the validity of secondary diagnoses.
3. The question of reporting consanguinity or its absence also seems worthy of note. An unsupported statement that the parents were not kin means little and is difficult to evaluate. If pedigrees could be extended far enough, practically every couple would be kin in some remote degree. It would seem logical to report consanguinity by specifying the degree of relationship that can be definitely excluded on the basis of reliable pedigree information.
4. For any population study it seems highly desirable that data should be reported in such form that later workers may perform different types of analysis in the light of later developments. Additional light may be shed on many problems by combining sets of data from various investigations, but this can be done with confidence only if the original studies are well planned and reported in some detail.

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DISCUSSION

Moderator: DR. CURT STERN, University of California.

STERN: Dr. Greenberg and Dr. Cochran have consented to discuss from a biometrician's angle some problems raised by the speakers.

DR. B. G. GREENBERG (*U. North Carolina*): The way in which Prof. Cochran and I would like to discuss these papers is for me to consider the basic items presented and the questions proposed. After discussing these, I should like to give

my own paper on the classical method of solving this problem. Prof. Cochran will present some of the newer ideas of sampling that may be applicable to the present problem.

The main problem considered by all the authors involves the concept of some kind of rate. This was expressed by Dr. Neel who was interested in the relative frequency with which certain of these traits occur. Any rate essentially consists of a numerator, in this instance finding all the cases available, and a denominator which in this problem is the population exposed. In such a procedure, there can be errors in the estimation of the denominator as well as the numerator.

The authors have concerned themselves primarily with that of the numerator. Dr. Neel recognized the incomplete registration in the numerator because of the difficulty of diagnosis and location of the cases. The incompleteness is more important than a sampling error and must also take into consideration incipient cases which have not yet been diagnosed as well as those which were misdiagnosed (false negatives and false positives).

I am a little concerned about what should be the true population for the denominator. This may be difficult to define when the accumulation of cases covers ten years. In Dr. Herndon's illustration it was twenty years.

The correct definition of the population depends upon what type of estimate is intended by "relative frequency".

There are two terms used in biometrics, one called incidence and the other prevalence. If relative frequency is defined as the number of new cases discovered, incidence is implied and the population is selected upon that basis. In contrast, prevalence is the relative frequency of the total number of cases alive at any one particular moment, and includes the accumulation of all new cases less those who recovered or died. In the genetic problems discussed today none recover, but they do die and move in and out of an area under study.

Applying this to retinoblastoma, births were the population and incidence was the type of relative frequency implied. It would have been better to relate cases to the year of birth, since these were known, rather than to the year diagnosed. Furthermore, the population or denominator was overestimated by about 5% since that many births die before reaching the average age of detection of two years.

In neurofibromatosis, the prevalence of this diagnosis was under study. The investigators found 16 cases in 4 mental hospitals since the disease is associated with some sort of mental retardation. Then, in studying some families with the disease, 213 persons with neurofibromatosis were diagnosed, only one of which had gone to one of these mental hospitals. The tenuous, and perhaps untenable, assumption was made that the hospitalization rate was fixed and that every case which belongs in a mental hospital was there. Thus, for each of the 16

persons in a mental hospital, it was presumed that there were 212 persons at home who never went to the mental institution.

The first question is whether mental retardation is associated with this disease if less than one-half of one percent of the cases is institutionalized for retardation. Is this very much greater than mental retardation in a normal population? I would imagine that it is not.

If one grants these assumptions about the constancy of the rate of hospitalization and considers only the sampling error, the minimum number of cases in the state is equal to about 1000 and the maximum has no real upper bound. This seems reasonable because of the small percentage who went to the mental institution. Knowing that there were at least 1000 in the state may not help much since there were at least 200 to 300 on the register already as a result of the investigation with certain families.

I should like to object strongly to the second method by which the same number of persons with neurofibromatosis was estimated by hospital data. There were 88 cases in the University Hospital; 10 cases in another hospital located in an average community, one of which was among the 88 in the first instance. Reasoning that for every case in the University Hospital there are 10 cases in the local community, the figure of 880 was arrived at. This figure was doubled for one reason, and then doubled again for another until it reached approximately 3400. Such reasoning is somewhat spurious and involves a large number of doubtful assumptions. I would question the validity of the result, or that it verifies the previous estimate.

I should like to point out in reference to Dr. Stephen's studies that the usual problems of sampling are not present. His problems are more concerned with exploratory work in estimating factors such as the segregation ratio, and others of concern to the human geneticist.

I would question, however, the results of his study on cancer of the stomach, particularly in the absence of controls. The selection of the probands may have been satisfactory but the use of the vital statistics rates of the general population is not a valid control in this instance. There is a need for considering age, race, and sex of the population as well as the limitations in published vital statistics data. This afternoon there will be an entire session devoted to probands and controls, and there is little point in discussing this further now.

In Dr. Herndon's studies, the problem of sampling does not appear to be the vital issue. He is essentially interested in group comparisons in a population which is almost completely enumerated. The precautions taken in drawing inferences were notable and there is little to be criticized. There was one difficulty in the sample of 10 counties selected for the study of blindness. This was a judgment sample or more probably, a chunk or convenient sample. As indicated, the investigators had hoped to increase the number of counties but were prevented from doing so. If a probability sample had been chosen, this problem might not have been so severe.

The authors have thrown out a challenge for a cheap yet efficient method of measuring the relative frequency of these uncommon traits. Unfortunately, there is no cheap and reliable method. If such a method was readily available, this problem would not be under discussion today. More expenditure of time and facilities will be necessary, and this is true of most research problems.

There are two alternatives available. Professor Cochran will discuss sampling techniques and what they have to offer. The other procedure is the standard or conventional one used notably in England for studies of relative frequency.

The person ascertained with a trait under study is called a proband (from German) or a propositus (from English). The procedure is based upon the assumption that the proband has been found in something like a Selective Service examination and not because he came to some center for treatment of the disease under study. By studying the sibs of that proband, one may find that no one else in the family has been located with the disease, or one may find that all sibs have, or any intermediate condition. I presume that many of the cases being discussed today would fall in the intermediate category.

In the instance where no other case has been found in the family by the ascertainment method being used, it is called single selection. In the case of all affected sibs being located, it is termed complete selection. The intermediate category is incomplete multiple selection.

In 1934, Fisher pointed out that the method of analysis depended upon whether there is single, incomplete multiple, or complete selection. The correct analysis in the case of complete selection is referred to as the Direct Method. It is exactly the same as when there has been a random selection of families for which one can use the binomial with the first term missing.

In 1938, Haldane considered efficient methods of estimation when single selection occurred. He proposed the use of what is called the simple sib method of analysis. This was a special case of the proband method in which one counts each family only as many times as it has been ascertained independently. In the sib method of analysis, each family is counted as many times as the number of abnormals which it contains.

The method of analysis for the cases of single and incomplete multiple selection follow the work of Haldane or Bailey, who in 1951, generalized the solution by quasi-maximum likelihood.

These are the conventional methods of ascertainment and analysis of relative frequency when one can afford to obtain reliable data. I recognize that it is an expensive procedure but we must face the fact that good data can not always be obtained cheaply.

PROF. W. G. COCHRAN, (*Johns Hopkins University*): I take it that one object of this meeting is to interest the statistician in the sampling problems faced by geneticists. This is a worth-while venture, for during the past 15 years knowl-

edge of both the theory and the practice of sampling has increased greatly. How much these developments can contribute to research in human genetics I do not know, but we certainly will not find out until the statistician, through close cooperation, clearly understands the nature of the problems faced by geneticists and the alternative courses of action that are open to him in coping with these problems.

It may be that progress will be slow. It has been my impression in scientific research that until the problems of measurement reach a certain stage of solution, the scientist is preoccupied with these problems, and it is hard to interest him in questions of sampling. I mention this point because in the interesting papers that were read this morning, the speakers seemed to be more troubled by problems of measurement than by problems of sampling, although perhaps the examples chosen were not entirely typical of research in human genetics.

I would like to speculate, in general terms, about a number of ways in which the advances in sampling methodology might contribute to research in human genetics. I doubt whether there are any broad, ready-made approaches that can be handed over to the geneticist as an answer to his prayers. For estimating the frequency with which some attribute occurs in a population, there are four general methods of attack.

1. If the measurements are cheap to make and the frequency is not too small, some kind of probability sampling is practicable. (The name "probability sampling" is used nowadays to include all the variations of random sampling that have been developed).
2. If the frequency is very low, but the attribute is relatively easy to locate and measure, we try to obtain a complete list of all members of the population which possess this attribute.
3. If the frequency is very low but the attribute is expensive to locate and measure, the problem is difficult. We may try to find some part of the population (e.g. hospitals) where the attribute will be concentrated, so that we can get some idea of the frequency in this area of concentration. We must then relate this frequency, as best we can, to the frequency in the rest of the population, as illustrated by Dr. Neel's example of neurofibromatosis.
4. For the previous case there is another method that has occasionally been feasible. This depends on finding a cheap presumptive test that has very few false negatives, although it may have false positives. This test is applied to a large probability sample in order to pick out a much smaller sample of presumed possessors of the attribute. In the small sample, it may then not be too expensive to use a more precise measuring process which definitely tells whether the attribute is present or not.

To judge from the papers presented this morning, I presume that these different approaches, except possibly the last, are already known in human

genetics. However, we are learning more about the scope of each approach and are able to choose between them on a sounder basis. There has also been some study of the important issue of deciding how much of one's resources should be devoted to problems of measurement and how much to problems of sampling. If one set of problems is entirely neglected in order to concentrate on the other, results are likely to be poor, and there is need for a proper balance between measurement errors and sampling errors.

In planning a survey, it has been found salutary to try to decide at the outset what degree of precision is necessary in the results, and then to design the survey so that this specified precision will be obtained. It is not easy to make up one's mind as to the precision required, but the effort forces us to think carefully about the uses that are to be made of the results and it gives a much stronger justification for incurring the cost of the survey, should this turn out to be too high. The alternative method of getting some data, making the estimates, and then asking "how can I compute their standard errors?" is rather haphazard and unbusinesslike.

In applications of sampling to sociology and medicine, there has been a trend away from the retrospective study towards the follow-through study. At first sight, the retrospective study looks attractive because data can be accumulated cheaply and without delay, and for this reason it is likely to remain a tool for obtaining results from which we can form initial hypotheses or speculations. But for a thorough examination of casual patterns, the slower and more painstaking method of starting with a group and following them through time is sometimes the only way of making substantial progress.

Sampling theory may also help by solving some specialized problems that are faced in human genetics. There is, for example, the problem of making estimates from groups of people who are genetically related, and of finding out what kinds of groups yield information at the lowest cost. Haldane's work in showing how to cut down the number of generations required for estimates of linkage is another instance.

Finally, since Dr. Stern assured me that the discussion was to be informal, I would like to make one suggestion without knowing whether it is feasible. From time to time, I have heard a geneticist remark that he would like to estimate the frequency of certain traits by means of a random sample, but he has felt that his own interests were not sufficient to justify the large cost of random sampling. However, a number of geneticists with interests in different traits could get together and jointly plan a kind of exploratory genetic survey which would obtain at the same time the kind of data in which each one is interested. The point is that the cost of the survey does not increase proportionately with the number of traits investigated. Moreover, a stronger case can be made to fund-granting bodies if the data will provide information needed by a number of first-class scientists rather than by just one.

DR. NEEL: I should like first of all to thank the discussants for their comments. It looks as if we are quickly establishing the atmosphere of give and take which some of us associated with the planning of this meeting had hoped to see materialize.

After listening to Dr. Greenberg's remarks, I wondered whether I had been sufficiently explicit in my own. I certainly did not intend to present our attempts to estimate the frequency of multiple neurofibromatosis and multiple polyposis of the colon as models of how such estimates should be made. Rather, it was my intention to describe in all frankness certain approaches we had pursued, and where they had proven inadequate. In this connection, perhaps Dr. Greenberg, as a non-geneticist, does not appreciate quite where we stand today with respect to the problem of estimating the frequency of certain inherited traits. We are not concerned with the second decimal point, nor even with the first, but more with obtaining an estimate which we recognize may be off by a factor of two or even more.

Now for several concrete points. Dr. Greenberg says he sees no "upper limit" to our estimate of the frequency of neurofibromatosis. I wonder if he would care to explain what he means by that. Don't the results of surveying an institutionalized population set an upper limit? In this connection, I should like to repeat that an estimate based on institutionalized persons is certainly an overestimate, because of the mental retardation seen not infrequently in this disease (Borberg, 1951; personal observations). Dr. Greenberg in his comments felt that the frequency of institutionalization in our series of cases with neurofibromatosis (0.5%) was not unusually high. I wonder if he was confusing the figure for the frequency of institutionalization for mental defect or epilepsy with the frequency of occurrence of mental defect or epilepsy in the population as a whole.

Secondly, accepting the inadequacy of the estimates I have presented, is there any way the biometrician can give us concrete help in assessing the errors of our estimates? Or, is this the kind of situation which defies attempts to calculate even the approximate magnitude of the errors involved? If so, a clear statement to that effect would be helpful.

Finally, I wonder if Dr. Greenberg didn't misspeak himself in his discussion of ascertainment when he referred to "single ascertainment" as implying that ascertainment was through an affected person, with no one else in the sibship likewise affected.

GREENBERG: I should like to elaborate upon the question of an upper bound in the case of Dr. Neel's problem. My statement should be qualified with the phrase "that for all practical purposes". Obviously, the total population is one number which represents a real upper bound. Unless one can introduce ancillary

information or material, there is no real upper bound within any practical range.

In referring to his other comment about methods of ascertainment, I did not mean to imply that single selection meant no other sib in the family was affected. According to the method used, single selection is to indicate that the chance of ascertaining independently another case in the family is very rare, not that there is no other person affected.

DR. C. M. WOOLF (*University of Utah*): Dr. Greenberg's criticism that the general population does not serve as an adequate control for the stomach cancer study perhaps needs further comment. For a cancer study of this type there is no question that it is difficult if not impossible to obtain accurate controls. It is a matter of using what materials are available and then evaluating the results accordingly. In the study which I carried out it was proposed that some insight could be obtained into the heritability of stomach cancer by comparing the death rates due to cancer in the sibs and parents of 200 stomach cancer propositi with mortality rates for the general population of Utah (1910 to the present). The causes of death for the relatives were determined by examining death certificates. The method of comparison used by Penrose, MacKenzie, and Karn in England (1948) in their breast cancer study was partially followed in this study, i.e., expected rates were calculated by taking into account the year and age at which the relatives died. As Dr. Stephens reported, the results showed that deaths due to stomach cancer in these relatives occurred at an increased frequency ($P. < 0.001$) but other types of cancer deaths did not occur any more frequently than expected in the general population. Calculating expected rates for different years and different age groups and comparing them with observed rates, does offer a method that can lead to the obtainment of worthwhile information. Further application of the method to the spouses of a large series of stomach cancer patients as well as to a control sample, in an attempt to partially check social and economic factors, has given results which support the usability of this method. The method has also been used to good advantage in a breast cancer study carried out concomitantly with the stomach cancer study, however other control measures were utilized as well. From the overall results of these studies carried out in Utah, I believe that the method has served the purpose quite well.

DR. PAUL R. DAVID (*University of Oklahoma*): The difficulty is that the index cases and their relatives are not likely to include proportionate representation of sections of the population differing in socio-economic level, rural vs. urban residence, etc. Rather, they will disproportionately represent those sections

in which stomach-cancer rates, or diagnosis rates, or both, are highest. Consequently, unless the general-population material is further subdivided into groups matched with the cancer relatives in respect to all substantially relevant variables, it can hardly provide competent control data, even if the effects of secular trends are eliminated.

DR. J. W. GOWEN (*Iowa State College*): Two problems appear important to this discussion; (1) rate of occurrence of a condition and a confidence interval for it, and (2) use to which the information is put after it is obtained, illustrated by the comments on mutation rates.

Rates are calculated as the cases showing the condition divided by the population in which the cases appear. Meaningful figures are hard to obtain particularly for human data. Indistinguishable phenotypes may arise as consequences of different gene actions. The same gene may produce different phenotypes under different environments or as consequences of different gene modifiers. The sporadic appearance of mutations in human pedigrees makes it nearly impossible to positively identify genes as alike or as just those having similar effects. The combination of data from different pedigrees to form the numerator of any rate calculation is subject to the same errors as those that could occur in combining amoebic and bacterial dysenteries. If the condition is genetic it may be due to several genes the mutation of any one of which may produce the observed phenotype. A case in our own experience is pertinent. Dr. Hollander discovered a gynandromorphic mouse in an inbred line reproduced brother by sister for some 35 or more years. Necropsy examinations indicate that less than one in one hundred mice of this line show the condition. The families of the gynandromorphic mice have sibs showing a less incidence than the families within the whole inbred line. It could be argued that this condition is not hereditary. Yet since the discovery of the first case, 39 other cases have been observed, all of these cases in this same line of mice, dispite the fact that 5 or 6 times as many mice originating from 14 other inbred lines have received equal attention. Heredity is certainly playing a very considerable part in the gynandromorphic condition. But how can a figure be assigned to represent the gene action? Human genetics has many like situations making for a dubious numerator in the rate calculation. The denominator of the fraction, the population from which the sample is drawn, is beset by even more difficulties. There is less to work with in estimating what the population should be. When data are combined from different pedigrees the sources of error are so multiplied as to invalidate the hypothesis behind the estimation of the confidence limits.

Mutation rates were discussed because their problems are pertinent to the use of rates in the larger problems of human genetics and science in general.

Inability to estimate either the numerator or denominator of the rate seriously interferes with any extended use of the rates to formulate broad generalizations.

DR. NEEL: I think that those of us who are interested in human mutation rates, which was not the subject of our discussion this morning, are pretty well aware by now of many of these pitfalls (cf. Neel, Amer. Nat., 86: 129, 1952). Perhaps during some symposium we can find time to take this up in the detail which it deserves.

GOWEN: May I return to the mutation rate question. In the human it may well be that the data do not represent gene mutation at all. They may be due to infrequent gene segregations and recombinations or other such mechanisms by which rare recombinants are known to occur.

DR. B. GLASS (*Johns Hopkins University*): Certainly the primary problem of ascertainment or measurement, as Prof. Cochran put it this morning, is the question of determining how many different genetic loci are capable of producing by mutation the same sorts of phenotypic effect which in our ordinary classification are confused with one another. It seems to me that the analysis of pedigrees is a major technique—in fact, the only ready way—to get at an analysis of that question. Because if we find that two different entities which clinically appear to be identical are inherited in distinctly different ways, or if by linkage studies we can show they have linkage to quite different genes, then it does not matter how much they look alike—to the geneticist they are quite distinct. That of course enters into the problem of the calculation of mutation rates, too. Thus, by our pedigree studies to determine mode of inheritance and linkage relations, we can get in some cases an answer to the question whether we are dealing with a single genetic entity or several that mimic each other. At the recent Genetics Congress in Bellagio, Dr. H. Harris told about his studies on cystinuria, which has in the past been described as a simple inherited mendelian trait. Yet when he came to study it in the laboratory with biochemical methods, he found not one but five or six different types of cystinuria that are distinguishable. Even so, we still don't know whether these different types of cystinuria that can be distinguished by laboratory or biochemical means are genetically distinct. It is possible for the same genetic entity or syndrome to vary in expression or in degree from situation to situation because of different modifying genes or different environmental factors that might be associated with it.

That leads me to make one other remark. It seems to me that in pedigree studies, one of the most important things to do and one strangely neglected to date, is to make a careful study of the variance within pedigrees and to compare

that with the amount of variance between different pedigrees, that is, with the variance of the trait in the population at large. If the condition is at all rare, so that you can be sure you are dealing with the same entity in both cases, we might in that way be able to determine to what degree the modification of a syndrome is due to modifying factors or environmental conditions.

DR. JAY L. LUSH (*Iowa State College*): In animal husbandry work with a case where the genes are so many that we can't identify them, we get almost nothing out of going more than one mendelian generation away from the animal whose hereditary worth we wish to evaluate. Sometimes it is worthwhile to go two generations away, if hereditability is low. That is almost the outside limitation. To go much further than that is only to waste effort. At the other end of the spectrum, where we are dealing with one single gene which we want to locate, it is frequently worthwhile to penalize individuals two mendelian generations away from the propositus or known carrier of the gene. In a problem such as recessive dwarfness in cattle, we do get a little bit in our selections by penalizing even the half sibs of the parents of a dwarf. Two mendelian segregations from your propositus is as far as you can get anything for your efforts at estimating whether the gene is present. When tracing a dominant gene forward, you might sometimes get some information by going 4, 5, or 6 generations down. But in that case it is difficult to discount the selectiveness of your data. You usually start on that problem with an affected great great grandfather or something of that sort. But you probably would not have had your attention called to him if he had not had several grandchildren. If so, it is a little dubious to count affected offspring and all his descendants as valid evidence. How do you allow for the affected contemporaries who did not come to your attention because they had few or no affected offspring? This is the kind of thing we find in animal husbandry problems of tracing single genes.

SELECTION OF PROBANDS AND CONTROLS[1]

Moderator: DR. C. C. LITTLE, Roscoe B. Jackson Memorial Laboratory

LITTLE: This business of being a moderator is a difficult job. It is I believe a term that came out of old New England meetings. It is interesting to hear this discussion and contrast it with the smaller group at Bar Harbor some 20 years ago, and also to think of meetings on Eugenics in 1921, the Congress in New York, which was enlivened by a symbolic act by a French anthropologist, who decided to take a shower bath (it is not certain whether it was the first shower bath he ever took). The water was much too hot, he became panicked, was parboiled, and spent all the remainder of the Congress in the hospital. Eugenists frequently got into mental "shower baths" in that violent period, characterized by emotional efforts on their part, which led to T. H. Morgan's paraphrase of Eugenics as "the rape of Eugenia." It is a fascinating characteristic of eugenics that there are so many varied reasons for studying human beings, and that human beings themselves are revealing so many vital problems for study. We must, therefore, always be tolerant of the different points of view that are sure to appear. There is not only an interesting problem of genetic abnormalities, but an equal challenge to select proper controls. There is at the base of research the whole question of studying unbalance—those conditions which produce the "constitutional" diseases of laboratory mammals and of man. The interesting work in the sex mosaics of mice or other rodents is a good example of a field in which the laboratory mammalian geneticists should work with the practicing physician and with the eugenicist in trying to analyze this fascinating, complex internal balancing system that means *health* and the departures from that balance that mean disease and abnormality. I feel that all the way from the lethals that cause death before birth, or even before implantation of the ovum up to long delayed lethals, like cancer, we have a great range of material. It is going to be hard to say that any honest research effort is not worth while. I appreciate Dr. Neel's plea for kind treatment by the statisticians, but I also think that unkind statisticians will helpfully keep us aware of our frailties as geneticists.

This discussion will deal with selection of probands and controls. Dr. Macklin who is to present the formal paper, is known to all of you and you know how long and patiently and at times very courageously she has kept in this field of her interest.

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[1] Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

Methods of Selection of Probands and Controls[1]

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As I understand the purpose of this talk, it is less to inform you, than to become informed by the discussion which is to follow it. I shall confine my observations mainly to what I have found practicable in my own work; not thereby intimating that this is the ideal procedure, but indicating only that through the method of trial and error altered by each successive experience, I have collected my probands and controls according to a given pattern. The purpose of a study determines to some extent the method of selecting the probands and the controls. The two essentials of all good research of this type must, of course, be the prime requisites; all else follows as of secondary importance. These two essentials are (1) that the sample be a random one, unbiased in its selection and (2) that it be adequate in size so that a statistically valid answer may be forthcoming. This paper will concern itself with the first of these problems, namely, the acquisition of a random sample of probands and controls.

The purpose of my research project was threefold; to determine (1) whether there was any evidence for a genetic basis for breast cancer in the human female; and (2) whether there was any evidence for the presence of the milk factor; (3) the role played by physiological factors. Because breast cancer appears many years after birth, and because there have already been found to be present certain physiological factors peculiar to women with breast cancer, it was deemed essential at the outset to secure a personal history as well as a family history of the probands for the evaluation of the role of these physiological factors. This at once determined the type of control which I would have to secure for the answering of the problem.

Had I been interested merely in finding whether there was as much or more breast cancer in the close female relatives of the probands as was present in contemporary populations, I could have used as control data the vital statistics of the state of Ohio, were these available in the form in which I needed them. I shall return to this point a little later. I was desirous, however, of comparing the reproductive history of the probands with control women of the same age. Such data are not available in any volume of statistics. This meant that I had to obtain a control series of women on whom I collected the same information as had been gathered on the probands.

At this point I must mention that I felt that it was necessary to collect two types of controls for the breast cancer patients; (1) a group of women who

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matched in age, woman for woman, the breast cancer patients; and (2) a group of patients suffering from cancer of some type other than the breast. When the proband relatives were compared with the first set of relatives, I should receive an answer as to whether there was more cancer, and incidentally more breast cancer, among the probands' relatives than among the controls. This would not tell me, however, whether the possible presence of more breast cancer in the former group was indicative of inheritance of a cancerous tendency without any site specificity, or whether there was an inherited site for cancer, so that one should speak of hereditary cancer of the breast, or of the stomach, but not of hereditary cancer in general. To answer this question the second set of controls was taken. When the patient was a male, personal histories of course could not be used for comparison, but the families of such males could be used for the study of possible genetic factors for breast cancer. If the genetic factor was for cancer in general, the relatives of the breast cancer probands should have no more breast cancer than was present in the relatives of the cancer control series; but more than was present in the non-cancer control relatives. If the genetic factors were for specific types of cancer, there should be more breast cancer among the probands' relatives than among the two series of controls. These points determined the type of controls which I would use.

METHOD OF SELECTING THE BREAST CANCER PROBANDS

(1) The first requisite is of course, that there be an adequate diagnosis, by a competent observer. In the case of cancer, the observer must be a trained pathologist. Hence no probands were accepted until a pathological diagnosis had been made proving that the proband had the type of cancer under study. All specimens of cancerous tissue in the Columbus hospitals are reviewed by one pathologist, thus assuring uniformity in methods of diagnosis. As most of the patients have metastases, there is no doubt as to the fact that the growth in question is malignant.

(2) The second criterion to be observed in obtaining probands is that they must form a random sample of the proband population. The ideal way to secure this appears to be to take a consecutive series of patients from one hospital. This is more easily accomplished in the realm of designing a project than in the field of actual performance. Many factors enter into destroying the continuity of a series of patients. Since in a genetic study, the patient is merely an introduction to a family group, it is obvious that the patient must have a family, and that she must have a sufficient amount of information about the family to make the study not only possible, but rewarding. At once, this *desideratum* made imperative the elimination of a large number of breast cancer patients, namely, the *Negro* group. Their knowledge of their relatives is often limited. Moreover, many of their relatives may be living in the South where they cannot be contacted. They may be too illiterate to read and answer

the questionnaire which is sent to them. Hence the first large break in having the series consecutive came when the Negro element, large in Ohio, was eliminated.

The second condition preventing the series from being consecutive was the fact that even among white patients, many knew too little about their family history to make a study feasible. They may have been orphans, raised by strangers, thus knowing nothing of family background; or they may have known only one side of the family, since the father had died young, or was divorced from the mother, or had deserted her; or the patients may have moved from the state where they were born, and have lost contact with most or all of their relatives. Or as often happened, they knew the given names, but none of the married names, and none of the addresses of their relatives so that the history was worthless for further pursuit.

Third, some patients were the only representatives of their family, or almost the only one, on this side of the water. Two world wars had made them lose touch with their European family, and information on the living and the dead relatives was not forthcoming. Although I have received death certificates and hospital reports from Canada, the British Isles, Switzerland, Australia and New Zealand, most of the foreign patients came from Germany, Austria and Italy from whence records could not be secured.

Fourth, some patients objected to giving the time, either in the hospital or after they went home, for an interview. Sometimes, because of family reasons they resented the questions asked and would not cooperate. Occasionally, they would start the interview, which had to be stopped because of meal time in the hospital or the arrival of visitors, and upon the return of the interviewer, they would refuse to answer further questions, having been warned by relatives or friends that they were "letting themselves in for something", they were not sure of what. Since I could not coerce a refractory patient into giving an interview, such patients had to be written off as a loss.

Fifth, the patient had to be intelligent enough to answer questions. On several occasions the patients were of such low grade intelligence as to make even a personal history impossible.

Sixth, patients practically all of whose relatives living or dead were outside the state of Ohio although still in the United States had to be eliminated. I depend upon letters sent to all living relatives within the scope of my study for information about themselves. In the event of their not answering, I follow up the letter with a call by the field worker to secure information. Relatives who live outside of Ohio are usually too far away to be visited economically; hence if they do not answer, I have no way of securing the information. Moreover, death certificates can be obtained from other states, but they are much more easily hunted for in Ohio by my trained workers than they are in other states, where there must be an accurate date and place in order to secure information.

Seventh, some of the patients who come into the hospital are private cases. In such instances, not only does the permission of the patient for an interview have to be secured, but that of the surgeon also. Sometimes this permission is not granted.

Eighth, there was only one field worker for the project, and hence only one person who could contact patients outside of Columbus. Not infrequently, a patient who had been operated upon in our hospital would die of her cancer before the worker had chance to get around to interviewing her.

Such a list of causes makes the acquisition of a consecutive series of cancer cases impossible. Apart from these many interferences, the series of cases selected can I think be considered fairly representative. The names of all the women who attended the cancer clinic of Columbus, which is a follow-up center for all the hospitals in Columbus and Franklin Co., or who were operated upon in University Hospital, and who were not excluded for the above reasons, were used. Since many patients in other sections of the state come to Columbus, the families were representative of the population over the state. No attempt was made to select women with cancer in their family background, particularly, cancer of the breast. The family history was unknown to the investigator at the outset of the interview, and hence no selection was possible whereby women with a great deal of breast cancer or with little in their relatives could be chosen.

METHODS OF OBTAINING CONTROL DATA

The difficulties met with in securing an unbiased sample of probands are negligible as compared with obstacles encountered in selecting controls. Although I am aware of a number of different methods of selecting controls, I have encountered none that did not pose some problems in their execution. Some methods sound ideal, and easy to carry out when put on paper; but are beset with difficulties when put into practice. I shall go over some of these and point out what I find to be the impediments to successful prosecution of the project, and why I have not used them in my research. I shall then give the methods which I have used, which also have certain disadvantages, yet have proved to be the only way out of a series of dilemmas.

1. First may be mentioned the use of the *vital statistics* of the general population as a control. The method compares the incidence of cancer in general and of the specific type being studied, in the sample of the experimental population with that of the population of the state. It is essential that the vital statistics of the state from which the majority of relatives are drawn should be the data used. The proportions of each type of cancer as well as the proportion of deaths formed by all cancers vary from state to state, and from decade to decade within the same state. This use of state vital statistics at once offers an obstacle. The relatives of the probands were dying at periods of time before Ohio began to compile vital statistics. The source of information for

years before that is in the United States Public Health reports. The data on deaths in Ohio are not in a form that is usable. Thus for the year 1906, the data for Ohio are given for the state, minus the four leading cities of Cleveland, Toledo, Cincinnati and Columbus. The data for these cities are then not given as such, but are included with data from all other cities of the United States of the same size. The number of cancer deaths, or breast cancer cases which came from Ohio is unknown.

Even were I to choose some later year, and standardize all the data on the basis of that one year for the sake of comparison, I still could not use the vital statistics of Ohio. Thus when the *total* of all cancer deaths is given, the data are divided into deaths among white and Negro males and white and Negro females. But when the data are listed for deaths from each individual form of cancer, there is no separation into male and female, Negro and white. In view of the fact that the percentages of the total cancer deaths formed by the different types of cancer differ, sometimes widely, for the two sexes and the two races, it is clear that such data are of no value for my work. Especially is this true since I have dealt only with white probands and controls.

In the study which I am now conducting, namely, cancer of the stomach and intestine, the condition is more frequent in the male than in the female, and is listed more often in the white than in the Negro, although the latter may not be indicative of a true racial difference, but merely of difference of accuracy in diagnosis and recording for the two races. It is apparent that I cannot use the recorded statistics for the state of Ohio as my control data. It thus becomes imperative that a control sample of the population be obtained, and the data from them used as the standard against which the breast cancer relatives are measured.

2. The husband's family and a sister of the husband as controls for the proband's family and a personal control of the proband respectively have been used by Dr. Reed in his study of the genetic basis of breast cancer. This secures for the most part an unbiased sample of the population, and also a sample with the same socio-economic background as that of the proband. It has, however, disadvantages. First, it assumes that there is a husband, and in my series of about 285 breast cancer cases, there were 8 per cent who were single. Second, it assumes that the husband has a sister, which in the relatively small families found here in Ohio, is not always the case. Third, it assumes that the sister is within easy access of the interviewer to give a personal interview. Fourth, it assumes that the husband's family is one whose records can easily be obtained, which might not be the case. The proband's family might all be in Ohio, while the family of the husband might all have lived and died in Kentucky, where records are not adequate. Fifth, it assumes that the sister, if available, and with a family whose records can be easily obtained, is willing to cooperate. Sixth, it assumes that even without a sister to furnish a control *personal* history, the

husband's family can be used as a control *family* history. In my interviews with men I have been increasingly impressed with the fact that the average male knows little of his family; it is the women who are the archivists of the race. Calls on the husband require almost invariably evening interviews, and are most often not worth the time it takes to make the contact. When the original cancer patient is a male, I try to get his personal history from him, and the family history from his mother or sisters, if such are alive. When all possible controls have been obtained by this method, it still leaves an appreciable percentage of probands for whom no control has been obtained. What method will one use for the selection of these additional controls?

3. One worker suggested that the controls consist of a routine series of women who were being operated upon for gall bladder trouble in the hospital where the probands were secured. This might seem to furnish an unbiased example of controls, but a little thought will show that it represents a biased population. Their families might be representative of the general population, but the women themselves would not be representative of women in general. Gall bladder trouble is first of all more common in middle age, and breast cancer patients range all the way from their late twenties to 80 plus. Such gall bladder patients might have the same mean age as the probands, but the range would be much less. Moreover, such women are likely to have had children, since gall bladder trouble is said to be much more prevalent among women with several pregnancies to their credit than among nulliparae. This would unduly exaggerate the observation that breast cancer patients have fewer pregnancies than the general population, because the controls were selected from among women almost guaranteed to have had several children.

4. Dr. Murphy, who made a study of hereditary factors in cervical cancer, used women from a dental clinic as his controls. This would appear to be without objection and in some places might work very well. Dr. Murphy, however, found that he could not get enough by this method since he resorted to women from university clubs to supplement his quota of controls. Personally, I found this method of collecting controls to be the least efficient one I tried. The women were sitting in rows in the ante-room of the dental clinic, and felt embarrassed when I singled them out to explain the project, to ask for their co-operation, and to make an appointment. They not only did not respond well, but those who did often found it convenient to absent themselves from home when the interview was scheduled. After securing about three interviews over a period of several weeks I hunted elsewhere for my controls.

5. *Hospital patients* being treated for some condition other than cancer have been suggested as an excellent source of controls. This is the group which has furnished me with the largest percentage of non-cancer controls. The patients were selected by me, with no knowledge of their family background, merely because they were white women who had no history of tumors or cancer

and whose physical examination gave no suspicious signs of cancer. They matched in age a specific breast cancer proband. I feel that this age matching is important, where each control is paired with a similar aged proband, rather than merely having the two groups agree in the mean age. The range might be widely different in the two series of women, and this would influence the comparisons on all factors such as number of pregnancies, age of menopause, etc. It has been claimed that patients taken from a teaching hospital might well not be representative patients. This might be true, but the objection could hardly apply to their families, who have many of them died before the patient entered a teaching hospital.

Ideal as this method might be, it soon began to show evidence of disadvantages. First, interviewing in the hospital is not satisfactory, since the patient is ill; other patients in nearby beds can overhear the questions and answers; the patient does not have available the addresses of relatives whom I want to contact; the interviews in the morning are constantly interrupted for diagnostic or therapeutic procedures. In the afternoons and evening, the arrival of visitors interferes with the interview. This can be overcome by making an appointment to interview the patient when she is out of the hospital. Many of them, however, came from nearby towns, so that home interviewing meant increasing the cost of the project.

Interviewing in the hospital, although conserving of time and money, put all the burden of the interview upon me. My field worker who was trained for interviewing did not have the entree to the wards which I had. With each shift of nurses, she would have to explain over again what she was doing and why she was there. As the project got further under way, so that more and more office work, coding of information, direction of the project, etc., devolved upon me, the interviewing of hospital patients as a source of controls had to be stopped and other methods sought.

6. *Healthy controls.* I found it better to interview the cancer patients themselves in their homes, rather than in the hospital, for several reasons; some of the burden of interviewing could be taken by the field worker; the patient had addresses and names of relatives in address books, often had family Bibles with birth and death dates in them; the patient was no longer ill, and hence was better able to give a long interview. While out in their homes, we often met neighbors who were visiting them, who were willing to give an interview as controls. Thus we secured people who were in the same social and economic group as our probands, and whom we approached with no knowledge of their family background. Through these combined methods of interviewing I secured 131 of my 245 controls or 53.5 per cent.

7. These methods still left me short of controls. Odd as it may seem, the people who are well are not particularly concerned with giving up their time to a project of this sort. I resorted to a method which has its drawbacks. This

was to make a public appeal through the Columbus papers, in which the project was outlined purposely with not too much clarity so that I could avoid people responding to the appeal who had too much or too little cancer in their families. The point was made that the only criteria to be observed were that the women should be between the ages of 40 and 80, the age group in which I needed more controls, and should never have had any tumor or cancer.

Appointments were made with all who responded; many were rejected because they had failed to understand that a family history was essential; or because their relatives had almost all lived and died outside of Ohio; or because they said they felt they would be good controls because no one in their family had ever died of cancer. Actually, I could have accepted this latter group without too much fear of prejudicing the results in favor of too low a cancer incidence in the controls. I rejected them, however, because I did not know at the time what I later found out; namely, that the proband, whether cancer or control, knew on the average about only 25 per cent of the cancers which actually existed in their close relatives. They knew that an additional 8 per cent had had cancer, but knew nothing of the site of the disease. Of the remaining 67 per cent of the cancers proved to have existed in their relatives by death certificate or by hospital report the probands and controls knew nothing. Hence when the control volunteer stated there was no cancer in her family, on the average she was unaware of 67 per cent of the cancers that had really occurred.

If the patients stated that they volunteered because there was a "lot of cancer", they were accepted. In only two instances were their statements borne out; the rest proved not to be a "lot" of cancer at all. The objection to this method of securing controls was that they might have erred in two directions: 1) in volunteering because there was no cancer as far as they knew; and (2) because there was a lot. Analyzing this group separately I found that they had about the same cancer incidence as had the group selected by me from hospital and healthy controls with no knowledge of the family background. Eighty-six of the controls were acquired in this way.

8. Unfortunately, even these methods left me with not enough controls. I again took a group which might be criticized on the basis that they represented a higher socio-economic level than the breast cancer probands. This group consisted of women who either worked on the staff or were wives of the staff of Ohio State University. Every one was unknown to me, and their names were picked from the catalogue of the University. They were asked to co-operate because this was research conducted on the campus. Twenty-five such controls were accepted; some who were written to had had operations for tumors, or had all their family outside of Ohio, and hence were rejected.

This group of controls was not as ideal as it should have been from the standpoint of the personal history. Breast cancer patients have fewer children than the general population; so have University wives, so that the inclusion of this

group would tend to lessen the actual differences in this respect between the probands and the controls. From the standpoint of family history, however, and incidence of breast cancer or of cancer in general in their relatives, this group was better than average for the following reasons. The group being better educated, had more knowledge of their close relatives, both as to names and addresses, than the probands had. Their relatives on the average responded to our letter for further or confirming information better than the average. Hence, I was able to make a more complete family pedigree for the group of relatives whom I included than was often the case with the probands. Obtaining information on all the close relatives would bring to light a higher percentage of cancers if they existed than would be possible with some of the incomplete family histories obtainable from the clinic probands and the same social level controls. Moreover, although the controls themselves were on a higher socio-economic level than the average proband, their families frequently were no more affluent than were the families of the probands, so that the criticism of a different socio-economic stratum in the two groups was scarcely applicable to the families although it was to the probands and this small group of controls.

Finally, a whole second set of cancer controls was investigated as outlined earlier. This group of controls differed from the first in that the control proband had a cancer of some sort other than breast in every case. The family history of these persons, approximately 325 in number, was collected with the same care as were the other two groups. When the cancer control proband was a female, her personal history could be compared with that of the breast cancer probands. The entire group of these cancer control probands was drawn from exactly the same source as the breast cancer probands, namely, the hospital records, and their family groups were on the same average social and economic level. The data from these families agreed closely with the data from the non-cancer control group, and the conclusions reached by comparing the breast cancer families with the non-cancer control group were sustained by the comparison of the breast cancer with the cancer control series.

SUMMARY

The problems of selecting an unbiased and sufficiently large sample of probands are not always easy, and the probands must meet certain criteria before they can be included in any human genetic study, apart from the fact that they evidence the trait being studied.

The problems of selecting a series of controls are even greater, and as yet no ideal method which supplies all the controls needed, yet comes within the scope of complete practicability and desirability has been found by me. The largest single group of non-cancer controls was secured in various ways, all of which appeared to be satisfactory, in that the controls were selected instead of volunteering. No knowledge of the family background was possible at the time

they were selected. The volunteer group may be criticized because it may have been biased in favor of too much or too little cancer in the family. The university group may be criticized more on the basis of personal history than on family history comparison. The relatives of the cancer-control group form a definite part of the general population and appear to have no objectionable features as a control group.

The project described was sponsored by grants from the American Cancer Society, the State of Ohio, and the Department of Health, Education and Welfare, Field Investigations and Demonstrations Branch, formerly known as the U. S. Public Health Services, Cancer Control Division.

DISCUSSION

LITTLE: I found Dr. Macklin's presentation to be most instructive, interesting and sobering. I know we want to hear from Dr. Reed, Dr. Anderson, Dr. Oliver, and Dr. Lilienfeld.

DR. SHELDON REED (*University of Minnesota*): The Minnesota project was started in 1944 by Dr. C. P. Oliver. When I came to Minnesota I inherited this monster. As it was an important problem it seemed very worth while to go on with it. I did not go ahead with exactly the same plans originated by Dr. Oliver. I changed the control. It was immediately obvious that we were to have troubles with the control, and the original control was patients in the hospital for gall bladder disease. Thinking that possibly this was a group selected with a particular somatic type of disease condition we rejected that control. Eventually the control used was the family of the patient's husband, and I have more confidence in this system of controls because Dr. Macklin has found only about seven things wrong with it. We know that there are on the other hand a number of very useful aspects to it, so we were rather happy with the control selected and worked with. I think there are about five groups that have a very good body of data now for this one problem—Dr. Macklin's material, Dr. Oliver's material at Texas, Dr. Murphy's material, and Dr. Woolf's material—all of which now have at least 200 propositae and combined would be a considerable amount of information. Now it is possible that one might think that those five experiments should have about the same result. That would be a wrong conclusion for a number of very obvious reasons. In the first place we are dealing with breast cancer which is a peculiar thing to be studying genetically, as more than one disease is included in this term. Breast cancer certainly is influenced by factors other than direct gene action, such as socio-economic status and marital habits. There are going to be differences in these

five studies and it will be most interesting to see how they differ, and, from that contrast we may be able to get something useful.

Sometime after I started working with this, Dr. Elving Anderson joined me and has been working on the project ever since. The good ideas in it have been provided by him, and he has done most of the work. He will describe some results of this study and let you see how the control worked and biases we ran into. We were under no delusions from the beginning that we had a random sample. Our attempts were to discover the biases, and find how our data were prejudiced, how urban and rural populations compare and if possible to discover some of those variables which make analysis so difficult. For instance, early in the work we found a Jacobson's hook in the relation of age and cancer prevalence. There was a peculiar shape to the prevalence curve that might have something to do with the hormones of the patient. We certainly do not have a random sample. It is impossible to see how it could be random when so many restrictions are placed upon it. The selection, the process that has given us the final sample, is obvious in many instances. As another type of control we turned to the data from the cancer registries. The one in the State of Minnesota was established recently, endured for only a short time and the information certainly would not be of great help to us. There is one in Connecticut and one in New York State. The one in New York State seemed to be somewhat more suitable than the one in Connecticut. The best material for us is that, not yet published, which Dr. Harold Dorn collected in Iowa where a study on cancer incidence was carried out by the U. S. Public Health Service, and we are most grateful for the material which he very kindly sent us.

Dr. V. Elving Anderson (*Univ. of Minnesota*): A study of the genetics of human breast cancer was started at the Dight Institute for Human Genetics in 1944, and the first comprehensive analysis has now been completed. A major objective of the present analysis was to explore the limitations and biases encountered in some of the methods used for studying human genetics.

Selection of propositae. The propositae were women who were examined at the Tumor Clinic of the University of Minnesota Hospitals and for whom a microscopic diagnosis of breast carcinoma was available (a total of 539 propositae). The selection of propositae occurred at two different levels. One type of selection (which might be termed Type A) depended upon those factors which brought the breast cancer patients to the Tumor Clinic. The second kind of selection (Type B) resulted from those factors which made the Tumor Clinic patients available for interview and inclusion as propositae.

The group of propositae in the present study was biased by long survival and correspondingly low age at first diagnosis (the result of Type B selection). The group was predominantly rural (Type A selection). The proportion of single women was comparable with the proportion expected from the state

population. However, evidence from cancer registries and surveys indicates a higher incidence of breast cancer among single women. It might be concluded that the group of propositae is not a representative sample of breast cancer patients with respect to this point (probably as a result of the high proportion of rural propositae).

It is probable that the group of propositae is not a representative sample of Minnesota women with breast cancer. However, it is thought that the group forms a suitable basis for genetic research. The group is probably as representative as is possible in the absence of a state cancer registry. The detection of sources of bias has permitted analyses to determine their effect upon the results of the study. Also it is thought that the control group provides a valid basis for comparison, even though the propositae are a selected group.

Selection of a control group. The control group was composed of the parents and sibs of the husbands of the propositae. This type of control was chosen primarily in the hope that it would be comparable with the cancer group with respect to the variety of factors involved in the social-economic status (residence, habits of nutrition, fertility patterns, religion, and nationality). It is probable that the families of husband and wife are on the average more comparable than other possible pairing of families. Other advantages of the use of the husbands' families as a control are: (1) It is not necessary to find entirely new sources of information. (2) The control families were selected for by the marriage of the patient and her husband. This type of selection should not be directly influenced by a family history of cancer. (3) Data about the husbands' relatives will be of great value if a follow-up study is made of the children of the propositae at a future time.

Calculation of expected numbers of cases of cancer. Morbidity and mortality statistics were used to provide estimates of the expected numbers of cases of cancer among the relatives. For the dead parents and sibs proportional mortality rates for Minnesota (specific for age and year of death, sex, and site of cancer) were used. This method appears to be most useful in the study of parents, since death records are frequently the only available source of information.

A second method employed cancer incidence rates for Iowa (1950) and New York State, exclusive of New York City (1945–47). These rates were applied by a modified life table to the age distributions of each group of relatives (both living and dead).

The results of these calculations indicate that the cancer incidence among the control relatives is reasonable, thus providing evidence that the control group is suitable for comparison.

Results. The observed number with breast cancer among sisters of the propositae was almost twice the number expected by comparison with the sisters of the husbands. (A study of families of women who developed breast cancer

prior to 1925 showed a similar excess of breast cancer among the daughters of these women.) Further information is needed for the interpretation of the breast cancer rate among mothers. There was no evidence for an excess of cancers other than breast among parents or sibs. The data do not permit a conclusion as to whether or not genetic factors are primarily responsible for this familial excess.

DR. C. P. OLIVER (*University of Texas*): I want to make a few comments about the work we are doing and also to refer to some points the other three speakers have given already. I certainly agree with Dr. Macklin about the difficulty of working with indigent patients. In our own work we have tried to use indigent patients as well as another group as our probands. The indigent patients primarily were Negroes, but some were whites. It seems to be a waste of time to try to work their familial records. One can not be too certain about much of the family history. Siblings of some indigent patients have only a mother in common. Little information about the fathers is available. The indigent patient will know her mother but may not know where the mother lives. A patient sometimes can tell you the area of the State in which she believes her parents live. A little checking results in the knowledge that the parents have moved and no one knows the new address. Information collected from and about indigent patients is not a total loss. The medical and personal histories are compared with those of the other cancer patients.

We have one excellent group of patients who are probands in the study. These are the private patients of a surgeon in San Antonio who has an active part in the project. The living patients are used as probands. Records of those who have died before they could be interviewed are not taken into the study. Our records include some information which we can get only from the patients. Concurrently we use as probands two groups of noncancer patients of the surgeon. Women in one group have had papilloma; in the other group, they have had other breast tumors.

In Minnesota, as Dr. Reed told you, I started out with women having gall bladder disease as controls for the study. That was done after consultation with some of the researchers interested in cancer. For several reasons we quit using gall bladder cases. We have tried to get control cases from among women who were under the care of a doctor for conditions other than tumor or cancer. Difficulties began to occur with this group of controls. Some of the probands were patients of gynecologists and a number of them were found to have had tumors. They had to be removed for the non-tumor controls. At the present time we are still trying to use women who are under medical care because we believe that the interviewer can talk with them long enough to get information about illnesses their relatives have had. It is certainly true that many well

individuals do not care to recall illnesses in the relatives. Many are rather proud of the good health of themselves and their relatives.

I would like to say something about the type of family records being collected. The family history is made as complete as possible. We contact even the first cousins of the probands in the control group, the cancer, and the papilloma and other breast tumor cases. The patient is asked to tell what she knows about illnesses in her family. She gives the names and addresses of her sisters and other close relatives who are then contacted. The mother and father, if alive, are asked to give information about their personal and medical histories, and about illnesses including cancer and tumors or possible cancers and tumors in the families. The term cancer can be used in the questions because the surgeon tells his patient whether she does or does not have cancer. The main difficulty is likely to occur with the women who have benign tumor of the breast. Sometimes one of them is afraid that she has cancer but has not been told the truth. Verifications of illnesses among the proband's relatives may lead to a cancer in an individual whose doctor does not want to have his patient know it. Under such circumstances, the questions are changed accordingly.

Close female relatives of probands are questioned even though they are reported to be free of cancer and tumor. That is an essential step because one will encounter some cases of cancer which are unknown to most of the members of a family. In one family for example, a patient knew about four relatives with cancer but questioning of the family led to information about 31 cases. One of these was a maternal aunt of the proband. The aunt had been reported as a noncancer relative, but she had had a bilateral mastectomy. She had not told any of her relatives. We get verification of the normalcy of the relatives as well as of the abnormal conditions.

The other methods we use are very similar to those reported by Drs. Reed and Anderson, and repetition seems unnecessary.

I failed to tell you that we have had to select some control cases from among women who are not under medical care at the time. These have been women who as members of an audience have heard about the study and agreed to cooperate. One can expect difficulties with these cases because, as Dr. Macklin stated, some of the women want to cooperate because there is cancer in the family. Some do not offer to cooperate because they have no cancer in the family, and they believe that you want cancer relatives in your study. One woman, about 63, said that she would not be a good control because there was not a single case of cancer in her family. Actually several cases of cancer were found among her relatives and she just did not know about them.

Selection of cancer probands for the study is not a problem. Every patient who comes to the surgeon and who has breast cancer, as proved by histological examination, is questioned as a proband. A patient is omitted from the first

interview only if she will not cooperate. Fortunately we have very few non-cooperators because the surgeon helps to break the ground.

The mortality and morbidity statistics for our State are of little use to the study. The records for the more recent years are being prepared but one can not go to records of the past and get desired information.

In conclusion, I want to refer to a statement made by Dr. Anderson. I would like to ask him to explain in more detail what he meant. If I did not misunderstand, Dr. Anderson referred to the familial tendency as not being evidence for heredity as a factor in cancer occurrence. He said that environment or diet might be the effective factors. Certainly the non-hereditary factors may be the explanation of cancer occurrence; but if we discard heredity merely because we know that environment is involved in breast cancer, we can ignore heredity as a factor for many other conditions. We should seek means for determining how effective heredity and environment are as agents. Familial tendency is at least an evidence in favor of heredity. In studies of breast cancer, one tries to control environment by selecting control cases from among women facing the same environment as the cancer cases, in so far as the environmental factors are known.

ANDERSON: The difference in breast cancer incidence between the cancer and control groups in our study was not large. (An estimate of the sampling error indicates that the probability of a difference as large as the one found for sisters is about four per cent.) The information may not yet be equally reliable in the cancer and control groups, in spite of attempts to get complete information. It is highly probable that the development of breast cancer depends in part upon non-genetic factors. For these reasons, it is felt that the present data will not permit a clear statement as to the relative importance of genetic and non-genetic factors in human breast cancer.

DR. A. M. LILIENFELD, M.D. (*Johns Hopkins University*): Dr. Macklin's, Dr. Anderson's and Dr. Oliver's presentations have indicated the difficulties involved in the selection of probands and controls and what I would like to do is make some very general remarks concerning selection of probands and controls. These remarks will obviously overlap with those made earlier, and consequently my remarks may be somewhat summarizing in nature. Ideally, in most instances, when studying the genetic aspects of human disease one desires a group of probands that is a representative sample of the affected individuals in a population. Generally, the families of such probands would be studied and the prevalence of the condition among the family members would be compared with the prevalence in the general population or among family members of a group of controls who are considered to be representative of the nonaffected individuals in the general population. When these two groups are compared, age,

sex, and race and whatever other factors that are known or thought to have an association with the condition being studied must be taken into account. This is the ideal situation. But in actual practice, as was quite apparent from the discussions, particularly from Dr. Macklin's discussion of the earthy details of what epidemiologists would call shoeleather epidemiology, these probands are selected from a hospital or clinic population, since this is a readily available group for study. Comparisons are then made with estimates of the prevalence of the condition that has been obtained by other investigators or by a study of control groups which have been selected in some way that leaves some doubt as to whether or not they can be considered as representative of the non-affected individuals in a population.

I would like to briefly review some of the difficulties that are encountered when one attempts to make certain inferences from the comparison of these two groups selected in this "practical" manner. One important aspect that has to be taken into account results from the difficulties of establishing criteria for the diagnosis of the disease. I think we have to continually remind ourselves that there exists a great deal of variability among observers, or practitioners of medicine, in determining whether or not certain individuals can be classified as having a certain disease or condition. It is essential that the same definitions and criteria be used in both the proband and control groups. This influences the selection of probands and controls. A good example of this is supplied by the many studies on the familial aggregation of epilepsy. In most of these studies the prevalence of epilepsy among family members of the proband is compared with the prevalence observed among Selective Service Registrants. There is a certain amount of intellectual insecurity in making inferences from such a comparison since the criteria upon which a diagnosis of epilepsy is based are obviously different in the proband group from those upon which a diagnosis is made in Selective Service Registrants. This morning one of the speakers spoke of obtaining a "normal" estimate group from that obtained among Selective Service Registrants. One must be rather careful of the inferences that can be made from such a comparison, although, it must be admitted that such a comparison may provide suggestive leads.

Another aspect with regard to the selection of probands and controls is related to the fact that probands are selected from a hospital population. Most commonly the hospital is a teaching center, since this is the type associated with the medical school where such research is usually carried out. Hospitals, as we all know and we all too quickly forget, vary considerably in admission policies and there is a great deal of selection of types of patients admitted to the hospital. Generally these hospital patients do not, and that has been repeatedly emphasized today, represent the affected population. There are other factors that influence the admission of a certain group of patients to a hospital or clinic. One of the most important factors is that of the economic circum-

stances of the patient. If one selects a control group from a segment of the hospital population with other diseases and conditions, one must carefully consider whether the factors in selecting these groups for hospital care are the same or different. If they are different and are related to the conditions being studied, the inferences made from the comparison may be quite biased. In most instances it is difficult, if not impossible, to estimate just what these selective factors for hospital admission are and thus be able to determine how they may have influenced the groups that are being compared.

One extreme example of this type of situation occurred to me earlier today. Let us assume that we would select probands with congenital heart disease from the Johns Hopkins Hospital. Obviously, all the congenital heart cases in Baltimore come to the Johns Hopkins Hospital. A control group of individuals who came to the hospital with refractive errors could be selected and perhaps matched with probands by age, sex, race, etc. But, one does not know what selective factors influence the admission of the control group to the Hospital. On the other hand, those admitted to the Johns Hopkins Hospital for congenital heart disease are not selected in any way since they probably are the entire affected population in the city of Baltimore. The difficulty of making any inferences from a comparison of these groups is obvious. We can generalize by stating that the proband and control groups, and this is a commonplace statement, should be comparable in as many respects as possible. Many times, though, it is impossible to consider all the factors with respect to which these two groups should be comparable if they were not selected in some random manner, since the factor that is not considered is the one that may later be found to be related to the disease being studied. Such a case arose in one study of cancer of the cervix in which the proband groups were in the low economic segments and the control groups in the upper income segments. A later study from Denmark indicated that the frequency of cancer of the cervix increased with decreasing economic status. It is then necessary to take this factor into account in comparing the proband and control groups.

Another example that is not completely related to the selection of probands and controls but which does indicate a similar situation, is that afforded by Dr. Herndon's study on paralytic poliomyelitis in monozygotic and dizygotic twins. In that study you recall, he pointed out that there was a higher percentage of concordance in monozygotic than in dizygotic twins. This finding must be evaluated against two facts in the epidemiology of poliomyelitis. Only 1 per cent of all infections with the virus have paralysis and there is some evidence indicating that individuals affected early in life are less likely to become paralyzed with the frequency of paralysis increasing with advancing age. It is also well known that the incidence of monozygotic twins does not vary with birth order, while the incidence of dizygotic twins increases with increasing birth order. Therefore, the families with dizygotic twins have more

older sibs than the monozygotic families. Last year there was reported the results of a serological study on poliomyelitis which indicated that when there are older siblings in the family there was an increased risk of the younger siblings becoming infected. Therefore, if there were more older siblings in the families of the dizygotic twins, there is an increased possibility of the dizygotic twins having become infected from the older sibling at an earlier age and becoming immune, with less chance of developing paralytic poliomyelitis at a later age. This may explain the differences in concordance found. It is still possible that the differences found are true ones but it is necessary to take this additional factor into consideration in any analysis. I think this represents a situation that may occur quite frequently with a comparison of probands and controls. As our knowledge of the epidemiology of cancer and other diseases increases it will be possible to look at more factors in comparing proband and control groups.

To many of us it is getting fairly monotonous to hear ourselves and others criticize the methods of selection of probands and controls used by other investigators, without being able to offer some concrete suggestions or better alternatives that are administratively practicable. Apparently it is difficult to obtain a good method of selection that is at the same time practical and not too expensive. It seems to me that the big difficulty we get into in discussing the comparison of probands and controls is that we on the whole continue to neglect the population. This morning Dr. Greenberg used the word prevalence, which is the ratio of the cases to the population. In family studies the usual procedure is to compare this ratio obtained in the family members of one group —the probands—with that obtained in the control group of families. But what we do not know is how closely our samples represent the population. Thus, it appears that the solution to the problem, and I think Dr. Neel suggested this this morning, lies in starting with a known population from which it would be possible to select probands and controls. Dr. Neel said that it is very difficult to select and describe such a population since it requires a census and then to select probands and controls from it. I would like to suggest two or three possibilities that could be used by geneticists to obtain a well defined population. One thought that occurred to me is that the population served by medical care insurance schemes might serve the purpose. The Health Insurance Plan of New York City has 250,000 individuals enrolled in it. Here is a population that is well described. A lot of things are known about the individuals in this population. There is no selection with regard to medical care. Couldn't a group of geneticists working on similar studies get together and select their probands from this population and knowing the rest of the population, the total population or the unaffected population, sample that population for the control group? I would like to hear what you all think of the possibility of the utilization of such a situation in an attempt to obviate the biases that exist in the studies

presented earlier today. There is still another possibility. In Baltimore for a period of years a census has been carried out in the Eastern Health District of Baltimore, consisting of a population of 100,000–120,000. Such a census should provide a population from which probands could be selected by matching with hospital records, etc. Then, knowing the rest of the population, it would not be too difficult to select a representative sample for a control group. Admittedly, there are some difficult problems in doing all this. Nonetheless, I think the gain obtained by such a method of selection is that one will always be able to look at the control group and see whether it is representative of the population.

Another possibility is that provided by the morbidity survey that the Commission on Chronic Diseases is doing in several parts of the country. I think there is one in Hunterdon County in New Jersey. There are other areas where such surveys are being carried out. These might be utilized for genetic studies. I throw these thoughts out for what they may be worth. I do not know how adequate the Eastern Health District or the various health insurance plans may be for the studies but I think they would represent, in general, an improvement over what has been done. I hope that we will get some discussion from the group as to the possibilities of utilizing this type of material for studies.

DR. MACKLIN: I shall try to take up the points which I have noted, in order, discussing the matter of control populations, socio-economic factors, size of sample, etc. First, the matter of controls. As I stated this afternoon, most workers find it difficult to get controls. Dr. Anderson told us that he used the data from an extensive study in Iowa as a population control for his Minnesota study. This might be satisfactory for his study, but might be completely inadequate for mine. Obviously, one could not compare data from a state like Ohio with a large Negro element, with data from a state like Iowa or Minnesota where the Negro population would be much smaller. If the Ohio data are not tabulated for the two races separately, and I have used only white patients, I cannot use even the data from Ohio for my purposes. Dr. Anderson stated that data from different states varied in accuracy. That is true. They also differ because of differences in racial components in the various states, even though the accuracy of the data may be comparable. Thus, cervical cancer is the commonest cause of cancer among Negro women while breast cancer is the commonest among white women. Data from two states with widely differing proportions of Negroes will not be comparable.

I commented upon the fact that Dr. Anderson's study left him with too few controls, since there was not always a husband's family history for comparison with his proband's family. If I understood him correctly he stated that this was a good thing, since it kept the controls fewer in number thus minimizing the error. This is exactly the reverse of the truth. The more nearly the size of

the sample approaches the size of the population from which it is drawn, the more reliable is the estimate derived from the sample.

Dr. Lilienfeld has suggested that a control group could be ideally selected from some large insurance or health group in which every person is listed and known. This method he feels would give one a representative sample of the population. I am not sure that this method would give any better results than that used by me, or by Dr. Anderson. My objection to his method was that it still left one with controls to be found elsewhere. The controls which I selected had relatives scattered all over the state of Ohio, in rural and urban areas. The relatives of the probands live in exactly the same areas of Ohio, coming also from rural and urban areas. The two groups were representative of the middle class white population in central and southern Ohio. Since it is the relatives and not the controls which are being studied for the incidence of cancer, it is a little difficult to see how selecting either cancer probands or controls from a teaching hospital and from a cancer clinic will give me a prejudiced set of relatives in whom to study cancer incidence. If the presence of cancer in the family history, or lack of it, was a factor in choosing patients for admittance to the hospitals, Dr. Lilienfeld's objections would have more validity. There is no such basis for selection of patients. Even if there were, the fact that a patient on the average knows of only one third of the cancers in his immediate family, as shown by my survey of data gleaned from the interviews with patients, the basis of selection would be quite fallacious, and cancer would be appearing where there was thought to be none.

There is additional proof that the controls and cancer patients come from the same general population. Not infrequently we interview a man because he is a relative in one of our cancer families, and months later go back to the same house to interview his wife as a relative of one of our controls.

I mentioned this afternoon that I had two types of controls; namely relatives of women at present without cancer and relatives of patients with a cancer in an organ other than the breast. These two groups of relatives are very similar in the amount of cancer they have, and it would seem that they are representative of the population in general.

The socio-economic factor has been mentioned as one of the factors associated with the onset of cancers of some types. There is no doubt that one finds cervical cancer more frequently among the lower classes than among the better or more favored economic groups. This I feel is not because lower economic status induces cervical cancer, but because it induces other physiological factors which are effective in causing cervical cancer. Similarly, breast cancer may be more frequent in women of better social and economic status. Again this is dependent upon the fact that the economic status favors factors which favor breast cancer. Cervical cancer women marry much earlier, have their offspring much younger, have larger families, nurse their children much longer

than the average woman, and much longer than the woman with breast cancer. These factors are found also among those of lower economic levels; women from poor families are less apt to be educated through as long a number of years as the average; they are less likely to have good jobs because of this; they therefore marry earlier as a means of support, begin their pregnancies earlier, know less about planned parenthood, and hence have larger families, and being poor, nurse their children instead of having artificial food for them. But one can keep the socio-economic level fairly constant as I have done, and find that this is not the main factor, but the other factors which tend to be associated with it.

Dr. Oliver mentioned that they take every patient whether in the indigent group or not. The group of patients with whom I have dealt are by no means indigent. The purpose of the Cancer Clinic is to be a follow up center for all cancer cases that are operated upon in Columbus hospitals. I have excluded the indigent group because either they have no family, or have lost contact with what family they have, and hence are valueless for my study which is on the families of these people.

I mentioned this afternoon that I took all the information on grandparents, and first cousins as well as on uncles, aunts, parents and sibs. Dr. Lush remarked that the information obtained on people who were more than one Mendelian segregation away from the proband was not likely to be worth much. May I explain why I did this? I was asked to study the evidence for or against a milk factor. In order to do this, I had to have data on aunts related to the proband through both father and mother. Breast cancer is for the most part sex-limited. How could one judge that breast cancer was coming through the father's line, unless one studied his female relatives; he himself could not show if he had inherited the factors for breast cancer. Similarly, I studied the daughters of maternal uncles and compared them with the daughters of maternal aunts. If the milk virus is operative, the only relatives who should show an increase in breast cancer should be those who are related to the proband through an unbroken line of females, such as maternal grandmothers, maternal aunts, and daughters of maternal aunts. If, however, one found an increase in breast cancer among these relatives, and found a similar increase among the female relatives related through the father, it would appear that the evidence was not in favor of a milk factor. This sort of evidence could be obtained only by going to relatives further afield than those removed from the proband by one Mendelian segregation. While I was collecting the data on the female relatives, it cost little more in time and money to collect it on the male relatives also.

LITTLE: There are two things I want to say in closing. I wish to thank everybody for the discussion and for the excellent presentations that have been made. There are two ideas that have come to me pertaining to a subject about

which I know very, very little. First, I don't want to discourage the point of view of the Seekers for the Holy Grail of "Random Sampling." That is all to the good, but why not use a deliberately prepared population? Why not take a series of populations of gall bladder cases, or one of diabetics, or of arrested tuberculosis cases, or anything else, and find out the cancer incidence in them, to see whether when you have deliberately discovered "unbalance" you get certain characteristic correlations with cancer? I have an idea that any random sampling of "a human population" does not exist very well, because we do not know what a *normal* population is. I don't believe that any sample of 500, 1,000, or 5,000 will be a normal population. The general principles of selecting your population for a type or types is not a bad one. Why don't we create known populations of genetic types of mice? We can put in a definite proportion of controlled genetic types. Why don't we try different methods of selecting probands and controls? Why don't we make available more epidemiological populations of animals where variables are controlled, and compare criteria of heredity with those in which we can't control the genetic types already present. It is easier to tell what is in a "mince pie" when you see the components that go into it than to try and pick them out afterwards.

PROBLEMS CONFRONTING HUMAN GENETICISTS[1]

Moderator: Dr. Laurence H. Snyder, University of Oklahoma

SNYDER: The program which we will discuss informally this evening is "Problems Confronting Human Geneticists," which is what we have been doing all day and will continue to do tonight. For moderators—Dr. Little and others who have moderated and will moderate, there is no speech to be made. I have no intention of making a speech; there never was any intention that I make one and it is only courtesy to Dr. Little that he can stop moderating and I continue for him. I did discuss this question of problems confronting human geneticists with one of my colleagues to see if he had any ideas. He said that, so far as he was concerned, they consisted of such things as headaches and ulcers;—those were problems of human geneticists. I think of course that we should continue the questions we have been discussing, or any others. I would be happy to have you branch out with reference to these problems we have discussed today and those we will discuss tomorrow. They constitute what are thought to be the problems confronting human geneticists and we hope to have some discussion of them that will aid the Study Section in evaluating proposals and will aid those who are working in writing up good proposals. After all, those problems on the program should be the ones we would discuss most. If you want to discuss other problems outside of these—there are of course a number I know who will—I would be happy about that.

I think it has been quite obvious through the day that none of the things we have been talking about, or doing something about, are going to lead to a definitive genetic analysis of the kind of factors involved and I think we are all cognizant of the fact that over the years we have fairly well skimmed off the cream of unit factors. We have come perhaps toward the end of what can be accomplished by what we call the traditional, atomistic approach of working with sample genes-single gene substitutions. We are getting away from the rare pathological traits and into the more common pathological things as well as such things as intelligence, social behavior, and other traits that differentiate people. And so much of what we have been talking about and will be talking about will bear on some method of studying polygene inheritance. That means of course that we have a lot of problems confronting us in understanding the genetics, etc. of polygenes or multiple factors on a different basis than the simple way we have been going at it in the past. For those genes which are single substitutions, one of the big problems is their biochemical actions;

[1] A discussion session of the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

pin pointing them. There are many things waiting to be worked out. We must reach the time when we can express this in biochemical terms in the way we can now with the phenylalanine story. The identification of carriers that many of us have been working on is a problem that needs lots more attention and I would be glad if anyone would care to discuss it for a little bit.

The problem of paper chromatography—we are not always sure how to evaluate it. What are the possibilities in human beings? Are there possibilities of identifying the carrier of disease through such methods? If so, I should like to hear the problem discussed a little, because we in the Study Section have to pass on proposals along those lines and it is not always clear to us as to whether or not they have any possibility of accomplishing anything. I think a problem that we should sometime discuss is assuring the general public, physicians and educators that the mere fact of our establishing a genetic background for a condition does not eliminate the hope of therapy or training as a method of modifying that character. I run into it all the time. The lack of cooperation of many groups with geneticists is largely the fear that if we establish a genetic basis, physicians and educators are not going to be able to use therapy or education or psychology or some other means of improving attitudes, education, and disease.

One last thing, and I am throwing this out in the hope somebody may have something to say, is the matter of coming to some conclusion on the controversial question as to whether or not we are all going to the dogs because of our increasing load of mutations due to removing selection, natural selection in so many instances. We cure disease. People don't die as early as they did. Are we then piling up mutations? Is this terrible thing that people are writing and talking about happening to the world or can we believe, as I would prefer to believe, that this is a straw man—that there is no danger? I believe that the gene itself is not harmful, only the effects in a particular environment may be harmful. If we can save people's lives, by the same token we can save the next generation's lives, and the fact that they carry those mutations is in itself no definite danger. I imagine there was a time in human progress when persons were killed off because they had poor vision. We will continue wearing glasses. We will continue taking insulin. I know there is a great deal of feeling about this question.

I can think of a lot of problems facing human geneticists. I am not sure they are any greater than the ones on the program and I would be happy to have you talk it over informally.

DR. D. P. MURPHY, M.D. (*University of Pennsylvania*): To continue the cancer discussion, we have discovered that probands, over 65 years of age, are unable to supply satisfactory information regarding their relatives, in many instances. This applies to the total number of their relatives as well as the nature of the

illnesses of the relatives and the causes of their deaths. For this reason it is necessary to interview, when possible, at least one relative in each family related to the proband. This makes it possible to check all information collected up to that time. The interviews with the additional members of the family group not infrequently bring to light much new information.

DR. MACKLIN: There are excellent reasons for checking every death certificate, no matter what cause of death has been given by the patient. The first is that you know you have the best official record obtainable in all cases in which you cannot get an autopsy or operative report. Second, you obtain a basis for estimating the accuracy of the information given by the patient. It may seem futile to check a record when the patient has listed auto accident as the cause of death; yet in one instance we found that a patient with cancer of the rectum had listed his sister as dying in an auto accident. Checking the death certificate showed that she had been operated upon for cancer of the rectum shortly before her death. Suicide is often given as the cause of death; in three instances I found that suicide was on the death certificate, but the information was added that the patient had killed himself because of the presence of some type of cancer.

DR. ARTHUR G. STEINBERG (*Children's Cancer Research Foundation*): I agree wholeheartedly with Dr. Macklin. It is necessary to examine *all* death certificates, not only those for persons reported to have died of a neoplasm. I would like to add that it is necessary as well to obtain pathology reports for all major operations, not only for those alleged to have been for cancers.

I would like to offer an easier way to get death certificates than by writing to state boards of health. I write to the clerk of the town or city in which the death occurred. These people are very cooperative and will find the death certificate for you if you can estimate the year of death within plus or minus five years. Only after the town clerk fails, do I write to the State Board of Health.

Dr. Lilienfeld pointed out that fraternal twins, because of their relatively late birth order, are less liable to paralytic polio than are identical twins. He suggested that this might be a source of error in Herndon and Jennings' twin family study of susceptibility to polio. This point would have merit if (a) the investigation had been conducted by selecting all twins and then determining the frequency of paralytic polio among the two types of twins and (b) if the probability of exposure from twin pair to twin pair is equal to that between pairs. The first condition was certainly not fulfilled because the investigators selected only twin pairs in which at least one member had suffered paralytic polio. The second condition seems unlikely to have been fulfilled

because of the much greater similarity of environment between members of a pair of twins than between pairs of twins.

The relative immunity which Dr. Lilienfeld mentioned is obtained by exposure to the virus itself. In each pair admitted into the study at least one twin was affected with paralytic polio; it follows that at least the affected twin was not previously sufficiently exposed to the virus to have gained immunity to the disease.

It does not seem unreasonable to assume that the previous history of exposure to polio of the members of a pair of twins living in the same household would be very similar. Hence if one twin failed to have had sufficient exposure to gain immunity, it seems likely that the other also will not have had sufficient exposure unless, of course, their thresholds of exposure to gain immunity are very different due (in the case of the fraternal twins) to genetic differences. This is precisely Herndon and Jennings' point, the greater differences between fraternal twins as compared to identical twins are due to the greater genetic differences between fraternal twins.

It will be recalled that twin pairs were included in the study only if at least one twin had paralytic polio and if both twins were living in the same household at the time of onset of acute symptoms. Hence, it may be assumed that both twins were exposed to the virus at the time when one of the twins contracted the infection leading to paralytic polio.

I think we must conclude that Herndon and Jennings' study is not affected by the point raised by Dr. Lilienfeld.

DR. EVERETT R. DEMPSTER (*University of California*): I think you are incorrect. To whatever slight degree infection in two twins might be independent, concordance would be less probable if the condition were rare; it would be more rare in dizygotic twins because of immunity resulting from the early exposure to older brothers and sisters.

STEINBERG: It isn't that the investigators went out and took all dizygotic pairs and compared them with monozygotic pairs. They started with dizygotic plus monozygotic pairs and that makes the difference.

DR. LILIENFELD: In several states in which there will be about 5 to 7 thousand cases of polio, information is being obtained as to the presence of identical and fraternal twins in households with one or more cases of poliomyelitis. From this information one may be able to see if there is difference in the risk of polio between dizygotes and monozygotes in households of different sizes. With 5 to 7 thousand cases being investigated, we may get 200 or 250 twin pairs and it might be possible to analyze this material so as to be able to take into account the variation in size of household.

DAVID: I wouldn't venture an offhand opinion on the validity of Dr. Lilienfeld's suggestion regarding Dr. Herndon's data on polio in twins. But I cannot refrain from remarking that the data in Herndon's paper are so fully presented, if I remember correctly, that they can be re-evaluated, if need be, in the light of Dr. Lilienfeld's idea. Furthermore, as data from other studies of similar type become available, collation with Herndon's material will be possible and answers to questions for which his data alone may be numerically inadequate can be obtained. Presentation of data in such detail as to permit re-analysis of this sort is all too rare. I should like to applaud Herndon for the exemplary form in which he presented his material and to urge in general the vital importance of this aspect of publication procedure.

DR. R. E. COMSTOCK (*North Carolina State College*): I have been impressed by the emphasis that has been placed on difficulty of obtaining complete family records and it has occurred to me that there might be merit in limiting the family investigation to the immediate family or even to only a part of the immediate family. For example in the case of breast cancer research one might consider collecting information on only the sister nearest in age to the proband or control. The argument would be that in general sisters near in age would maintain close contacts so that this sister would usually be the easiest family member to get information on. The suggestion assumes that information lost by incomplete family check-up would be compensated, at less cost, by investigating more families.

The argument against the suggestion is that in the case of rare diseases, number of probands may be the primary limitation on scope of study so that one could not increase family number even if he wished.

There are two favoring arguments. (1) Cost of information per individual might be reduced. (2) As pointed out by Dr. Lush this morning, the value per individual of the information is reduced for relatives other than the parent or full-sibs. Another point that seems worth making is that when information on more than one person per family is used the simple chi-square significance test may not be appropriate. The reason is that this test assumes independent probabilities of the event, in this case occurrence of the disease, for different individuals within either of two or more classes being compared. This could not be assumed for individuals from the same family. I doubt if this issue is of much importance for diseases that do not run strongly in families though I cannot speak with authority on this point. There are many statisticians that could. On the other hand I'm quite sure that with diseases for which intra-family correlation is high the matter becomes of some importance though in cases where the intra-family correlation is really quite high it is probably a minor issue by comparison with the first two listed.

DR. A. S. WIENER, M.D. (*Brooklyn, N. Y.*): When pedigree data are collected, the results are apt to be colored by the preconceived ideas of the investigator. Therefore, a good plan is to have such case histories taken by two workers independently, and from different members of the family. If comparison of the histories shows satisfactory agreement, then the results may be accepted at face value.

Tests have been suggested which are supposed to detect genetic carriers of diseases such as sickle cell anemia, and hemophilia. The results obtained with some of these tests are not readily reproducible, however, and the observations are readily influenced by suggestion. For example, if an investigator knows he is testing the blood of the mother of a hemophiliac, he is apt to find her coagulation time prolonged, even though it really is entirely normal. To avoid such erroneous results, it is necessary to use the blind test, in which the observer has no way of knowing whose blood is being examined. Each subject tested should be presented to the observer as a numbered unidentified individual, rather than as a relative of a proband. If necessary the subject's arm should be presented through a hole in a wall, so that the individual drawing the blood sample cannot identify him.

I believe that by this time everyone agrees that almost any condition affecting the human body has a constitutional aspect. With regard to carcinoma of the breast, therefore, the question is not whether heredity plays a role, but how important the hereditary factor is, and whether this can be modified by controllable environmental influences. The role of constitutional factors is evident also in studies on immunity. Individuals exhibit marked differences in their capacity to form antibodies. Thus, there are rare individuals, who after multiple transfusions, have formed antibodies to several different antigens of poor antigenicity such as N, rh^w, Kell, Duffy, S, etc. The frequency of such instances of multiple sensitization is much higher than would be expected through coincidence alone, indicating that a constitutional factor is involved.

Individuals appear to exist with a constitutionally increased susceptibility to carcinoma. In such individuals, after a skin carcinoma has been successfully removed surgically, a primary malignancy of a different type may develop elsewhere in the body. The problem of leukemia is related to that of carcinoma. There is a dearth of information regarding the familial incidence of leukemia, though the occurrence of leukemia in members of the same family appears to be rare.

LUSH: The statisticians do not always require a random sample. Sometimes they recommend stratified samples or some other kind of sample which is not completely random. The important thing generally is only to make sure, when you pick within those eligible for controls, that you do not pick them in some

biased way. The characteristics which you are going to study in the sibs or other relatives of the control should not influence the choice of the control. To do otherwise would get the dependent and independent variables inextricably mixed. The statistician may well insist that within each stratum the sampling should be random. The second thing which may need saying here about statisticians is that we sometimes may imply too much confidence in the scope of their work by imputing to them a biological interpretation, which is really our own and which they did not really venture. For example, in the case first discussed I believe a 2 x 2 chi square test would be valid, but valid for answering what question? It would tell, with a certain degree of probability, whether the two samples could or could not be random samples from the same source. With that answer, I think the statistician would generally state that his job is over. If the sample could not have come from the same population, he would rarely, if ever, specify the biological reasons for the difference between the two populations. For example, this process of comparing the sibs of the propositus with the sibs of the chosen control is a matter of the correlation between sibs. The statistician may be able to tell whether it is or is not significant but he would hardly draw from that any conclusions about how much of the correlation was caused by environmental things and how much by heredity they had in common.

A third point is that the causes of mortality are often multiple. I once worked a short time for a poultry breeder who was intensely interested in the genetic aspects of all kinds of mortality in chickens. Every bird which died and even every egg which failed to hatch was posted by a veterinarian. Frequently the bird would be found to have several things wrong, any one of which would have caused its death eventually. We attempted once to apportion its death among the various causes, with some rough weighting according to the probability that death would have ensued from a given cause and according to the probable proximity of death from that cause. As a very crude example, if a bird were run over by a feed cart, but, in the post-mortem, was found to have leucosis of the liver and also bumble foot, we might have put it down as 50% dead of the feed cart accident, 45% dead of leucosis, and 5% dead of bumble foot! Naturally this attempt soon broke down, not only because it was so subjective but also because it led to many inconsistencies. Since primary interest at that time was centered on the problem of breeding for resistance to leucosis, we adopted the objective (but somewhat one-sided) criterion of marking the bird dead of leucosis if any leucosis lesions at all were found in the post-mortem, regardless of the immediate cause of death. This worked well enough if our sole interest was in leucosis but I dare say that it gave us a distorted perspective about the other causes of death. Likewise, I suppose that if a man is killed in an automobile accident and a post-mortem shows that he

had cancer of the stomach, your point of view is that he would have died of cancer if the accident had not happened?

DR. MACKLIN: We are interested in knowing whether the relative had cancer, not whether he died of it.

LUSH: I suppose that corresponds to the working point of view we adopted for investigating leucosis in chickens, but I am reluctant to share your confidence in these death certificates. My own father died in his sleep on a train. The coroner performed no autopsy, but the death certificate states that the cause of death was angina pectoris. That may have been accurate, but the coroner really made no effort to be certain. I wonder how many death certificates are filled out accurately?

DR. MACKLIN: You are quite right that the death certificate is not all that it should be. There are errors of both omission and commission. Nevertheless, a physician is not likely to enter cancer as a cause of death when there was no cancer; although he may sometimes err in the opposite direction of failing to enter cancer when it was present. Imperfect as the death certificate may be, it is still better than the unverified, often very inaccurate statement of the patient. I assume that the same amount of error is made on the death certificates of the grandparents or parents of the control series as is made on the certificates of relatives of the proband series who were dying in the same decade. Thus, if the probability was ½ in 1900 that a woman dying of breast cancer should have that fact entered on her death certificate, the female relative who died in 1900 had that same probability of error, provided that she had breast cancer, regardless of whether I picked up her granddaughter in 1953 as a control case or as a breast cancer proband.

LUSH: I suppose that can be considered as a statement of faith in randomness.

DAVID: I think that there is a good deal of merit in what Dr. Macklin says. In cases for which it is obtainable, I think a statement from the attending physician is highly desirable. Death certificates often do not reliably report the cause of death, and reliance upon them may lead to invalidating biases. If one were to investigate mortality in the State of Oklahoma and perhaps more particularly in Oklahoma County he would find tremendous mortality from acute congestive heart failure. They have the justice-of-the-peace-coroner system, and when an unattended death occurs, a justice of the peace writes out the death certificate. I don't know how many years ago a justice consulted a physician about possible causes of sudden death, but he evidently

liked the sound of the words "acute congestive heart failure" and that has been filed on an exorbitant number of certificates ever since.

I would like to applaud Dr. Lilienfeld for something he touched briefly during his discussion this afternoon, which I think deserves further consideration and reiteration. This was his suggestion that those who are engaged in human genetics research should investigate the possibility of making use of such areas of organized medical and epidemiological investigation as are embraced in the health insurance plan in New York or the Eastern Health District in Baltimore. The use of such areas would by no means solve all of the difficulties we have wrestled with in relation to sample selection, but it would diminish many of them and would provide, additionally, some special advantages. The population of the Eastern Health District was chosen, in part, because of its relative stability, and I believe it has been under constant survey by Public Health nurses and by epidemiologists at Hopkins for a considerable period. A great deal is known about the population. Morbidity data are regularly collected (I think there have been quinquennial censuses); and economic and occupational data, along with much other material that ordinarily would have to be obtained independently for any particular study, are in punch-card files. But we need more such areas throughout the country. Are there others? There should be one at least in each geographic quadrant; there should be one in the midwest. We need others to provide ecological variety, as well as to furnish an aggregate population large enough to provide samples of adequate size for investigations like many of those discussed today, particularly by Dr. Neel, for which a single area would not be large enough. The maintenance of these areas would admittedly be expensive. But with the same area providing material for a variety of investigations, epidemiologic, sociologic, genetic, etc., there should be a considerable overall economy, in that many basic data requisite to all of the studies would not have to be obtained separately for each. Perhaps even more important, follow-up would be possible at minimal cost. If we do not have opportunity to discuss this question further during the Conference, I hope at least that it will be kept in mind by all of us as an important one to explore.

DR. LILIENFELD: I think Dr. Macklin asked me a question when she answered some other questions. The question concerned the ability of being able to discover the influence of various factors on the incidence of cancer if a medical care insurance scheme was utilized for study. It seems to me that this type of population offers the best means of studying those factors. If we take a population and then define the individuals in that population by various characteristics, we can then determine the prevalence of cancer in groups of individuals with these differing characteristics. This type of population also permits the use of

anterospective studies. In these studies one could follow groups of individuals defined by different characteristics and then see if the risk of developing cancer differs in these various groups.

Dr. David asked whether there were more areas similar to the Eastern Health District in Baltimore. So far as I know there are no other areas similar to that. There has been no census taken there since 1947 and there is some question as to the value of having another census taken. I am certain that if certain population groups of this sort are started throughout the country it must be kept in mind that they are expensive. However, the medical insurance schemes and the health insurance plans are in existence for other reasons. You do not have to set up a special population. I think consideration should be given to working with available material. There are a lot of data being collected now and if a genetic study could be grafted on, this would be very fruitful.

GLASS: If a homogeneous group such as that could be used, it would contribute an additional advantage. I was glad when the inaccuracy of death certificates was brought out, because if you are trying to get the absolute prevalence or incidence of a condition, the death certificates are clearly going to be closer to the real rate than reports from people interviewed, but even so the death certificates are not going to be perfect. There is one thing about records taken from personal histories or reports from individuals in the sample that I think has not been brought out, and which makes that kind of data worth more than one would believe from what has been said here today. If the sample from which the individuals are drawn is really representative of the population, then two independent groups of individuals questioned about the same thing, even though their recollections are faulty, and even though some may lie about the matter, will yield results that can be shown to be practically identical if the samples are sufficiently large. We had an experience of that kind a few years ago in studying the records of the pregnancy histories of women coming into the Rh laboratory at Baltimore for blood-typing. Each woman was asked to report on her previous pregnancies, including miscarriages, abortions, etc. Some women don't recall too well what their past pregnancy record is. Many of them who have induced abortions will lie about it. You can think of all kinds of reasons why these histories would be very inaccurate. Yet when we took one sample of 1,000 women and summed up their reports and compared them with the answers from another sample of 1,000 the two break-downs were startlingly alike. So, if you were to take personal reports, of the kind we have been talking about today, from two different groups and you found a significant difference between what they reported, then it would show either that there was a real difference in what they were reporting about or else that there was

some systematic bias that made them lie differently about it (or recall it better or worse). If there was any real difference, the lying or forgetfulness wouldn't conceal it.

DR. GORDON ALLEN, M.D. (*N. Y. State Psychiatric Institute*): If one is inquiring into the family histories of a cancer group and a control group that are truly comparable with respect to cancer, it is, indeed, probable that distorted answers will not produce a spurious difference. But in the studies under discussion, this method of inquiry revealed no difference between the cancer and the control groups, so we are not concerned with a difference that may be spurious. It would seem relevant to point out, on the other hand, that if the cancer group has a higher incidence of cancer among relatives, a tendency to conceal such cancer will diminish or eliminate the apparent difference between the groups. Hence, where the method of personal inquiry was relied upon to show a familial tendency in cancer, we are probably justified in dismissing the negative findings as inconclusive.

GREENBERG: There are several places in the U. S. where genetic studies may be conducted in conjunction with other programs. The U. S. Public Health Service studied the population in Hagerstown, Md. for many years. The School of Public Health at Pittsburgh has started a sample survey to study problems similar to those conducted in the Eastern Health District. The National Heart Institute is studying a community in Massachusetts (Framingham) over a long term period to learn more about heart diseases.

I want to thank Dr. Lush for defending the statistician. As a representative of the biostatisticians, I am very glad that the faults and limitations in using death certificates and the data derived from them have been pointed out. The ones mentioned are the obvious inaccuracies. The term congestive heart failure, or heart failure itself, is not an approved term on death certificates today.

DAVID: It is in Oklahoma, Sir.

GREENBERG: I stand corrected. The justice of the peace and coroner system for the medical certification of death is a regrettable thing in all states where it is still used. In North Carolina, as many as 10 percent of the death certificates may be signed by coroners, most of whom are not qualified to determine the cause of death. I should like to ask Dr. Macklin if she got photostat copies of the death certificate, or did the vital statistics offices tell her the cause of death.

DR. MACKLIN: I get a photostatic copy from some states, sometimes a typed copy of the certificate; sometimes the cause and contributory causes of death

and date of birth and death are furnished. I obtain the Ohio data through my own workers, who note whether there was an autopsy, operation, etc., as well as main and contributing causes of death.

GREENBERG: Receiving the photostat or typed copy was a good idea because the medical certification has two parts, underlying causes and contributory causes. The condition which Dr. Macklin was seeking on the certificate might be listed under other significant conditions and not tabulated in the enumeration of causes of death.

Incidentally, the point which Dr. Macklin made about the bias in the vital statistics not affecting either one of her groups more than the other is a valid one. One may have a sample which is poor when used to estimate the frequency of some characteristic yet the sample may be an unbiased one for another use to which it is to be put, such as comparing two groups. For example, this group here today may not be a very representative sample of the population but if one wanted to determine if males were taller than females, the sample might be a valid one for that purpose.

There are one or two more defects with vital statistics data which may affect your studies. The data around 1900 are subject to errors in diagnosis of cancer. How many physicians knew how to diagnose cancer then? Furthermore, it was not the mode, or as fashionable, to have a diagnosis of cancer as it is today. Finally, the time of death is also important since deaths during that period were likely to occur before persons were old enough to have cancer.

I should like to ask Dr. Macklin if she has ever considered a separation of the propositae according to the grade of cancer by pathology. This is equivalent to an internal type of control. Do those with grades 1 and 2 have less relatives with breast cancer than those with grades 3 and 4?

DR. MACKLIN: We do note the histological grading.

I have not considered that of much importance for two reasons; (1) that the grade may differ depending upon what part of the tumor has been taken for diagnosis; and (2) the grade may and in many instances certainly does depend upon the time at which the material was taken for section. If early in the development of the tumor, the grade may be quite different from what it would be if taken months or years later when the tumor has existed for some time. It is a subjective standard, and not subject to rigid rules. We have the data, however, on most of the cancers and will be able, therefore, to see if there is any correlation between the grade of the cancer and the extent to which it appears in other close relatives.

The degree to which different cancers were diagnosed in past decades varies widely from cancer to cancer. Primary lung cancer was not often diagnosed 30 years ago; this may have been caused by lack of lung cancer then, or by lack

of accurate diagnosis. Fashions in diagnosis change; years ago, the organ in which the cancer was most evident or in which it seemed to do the most damage was listed as the site of the cancer. The liver is one of the commonest sites for metastases, hence cancer of the liver was frequently listed as the cause of death 25 years ago. Today, since it is necessary to enter the primary site of the cancer if it is known, cancer of the breast, stomach, or pancreas are more commonly found and cancer of the liver as a diagnosis is much less common.

DR. NEEL: Several years ago I attended a Milbank Symposium concerned primarily with family studies as a method of public health research (1951 Annual Conference, Milbank Memorial Fund). I went there expecting to meet a good many of my fellow geneticists here today, and was somewhat taken aback to discover I was the lone representative of our discipline. It was quite educational for a geneticist to see how many ways it was possible to study families in a non-genetic fashion, and also the magnitude of some of the studies in progress. I think it important that we realize that the epidemiologists and the physicians interested in health insurance plans who have been the prime movers behind many of these projects have a lot of very real probems they are trying to get at. Furthermore, the individuals comprising the populations under investigation in these family studies will not accept an unlimited intrusion into their private lives. Under the circumstances, I got the impression of considerable resistance to anyone—regardless of his interests—who wanted to step into some going study and graft one or two more questions on the questionnaire. I wonder whether we as geneticists—granting that the epidemiologist has quite a head start in the study of populations—aren't about ready to organize our own population studies. Such studies will be expensive, but are the only way to get a clear picture of the mortality and morbidity due to inherited disease.

DR. J. N. SPUHLER (*University of Michigan*): I would like to comment on the statements by Dr. Snyder and by Dr. David that the study of major genes, of characters with simple modes of inheritance in man, is almost complete. Now, if you mean by that that it should no longer be considered highly praiseworthy to study trait X in ten families and report as a final and exhaustive conclusion that "X is determined by a single dominant gene", then we would all agree. Reports based on untested inferences from a small number of pedigrees are still important but not particularly exciting. But, if you mean that the study of major genes from the point of view of formal, physiological and population genetics is nearly complete, then all cannot agree. It seems to me that the study of major genes in man is very incomplete. Yet this is the problem area we do know how to attack. Dr. Snyder mentioned the need to know more about

physiological genetics of man and to know more about the possible accumulation of deleterious genes in the population. These problems can best be attacked by taking a known unit variation, some variation that can be associated with a known set of genes or a marked chromosome. If we make our criteria fairly broad, there is not a single unit character in man that is well known genetically, including the serological characters, in terms of identified genes. For man we know practically nothing about selection, very little about mutation, very little on tested cases of genetic drift, very little about the breeding structure of populations. There is not a single case where the physiological action from gene to phenotype is completely known for man.

There are two fundamentally different ways in which we become interested in the genetics of human characters. If our primary interest is in the trait itself, and the trait is something like stature, then we must use methods suitable for quantitative inheritance, but if our primary interest is in human genetics, and the particular trait selected for study is secondary, then I would suggest that we need to spend another 50 years on characters with simple modes of inheritance.

DAVID: I would agree with that. I, myself, would not say and I do not think Dr. Snyder intended to imply that the study of simple modes of inheritance in man is complete by any means. There is still a great deal to be done on the problem of single-gene inheritance in man. I'm a little astonished, however, if I understood Dr. Spuhler correctly, at the suggestion that we possess a well-defined methodology for the attack on this problem, because when we had a preview of this evening's program earlier today, I thought there was rather unanimous agreement that there are many perplexing difficulties, still unresolved, in the definitive identification of single major genes in man.

I would like to say that I am pretty well convinced at the moment that the bulk of genetic variability in the human species, and I suspect in any natural (as opposed to laboratory) population of animals, is not referable to major-gene differences but is polygenic, and I think probably not analyzable in terms of single gene differences. In man, we have a strong basis for the conviction that most of the differences that distinguish individuals in superficial appearance are genetically determined, because of the almost perfect resemblance between monozygotic twins. Yet these differences have thus far almost completely defied all attempts at satisfactory analysis in terms of individual gene effects. This does not mean that it is futile to continue searching for such effects. In some areas, indeed, notably in serologic characters, the search has been highly productive. Of course it must go on, and in a variety of ways. But I think we can envisage a point of diminishing returns, beyond which further progress will require some sort of a revolution in our approach to the analysis of human variability. I haven't the slightest idea what form the revolution will have, but

I think we should be on the alert for it, and should make every effort to discern the direction it should take.

Another point for comment relates to the potential utility of chromosome markers. Unless some spectacular new discovery is made in human genetics—something at least comparable in its impact to the discovery of salivary-gland chromosomes in Drosophila—something permitting the ready identification of galaxies of new genes, it seems to me that human chromosome marking is not likely to have more than incidental utility. I have expressed this viewpoint before, but it has been more adequately and ably presented by Dr. Neel within the past few years (J. V. Neel 1949: Amer. J. Human Genetics *1*, 19–36).

SPUHLER: If you have trouble studying the simple cases then you are going to have much more difficulty with more complex cases where the variation is associated with, say, 10 genes. Another point is this: You mention that facial characteristics, and most other physical characters anthropologists are interested in, are controlled by multiple genes. Probably so. The distinction made earlier regarding two different reasons for being interested in a particular character is pertinent here. For example, if the basic problem is to determine the relationship of 2 populations in terms of some genetic model, then it is futile to work with traditional methods on traditional traits like stature, cephalic index, etc., when much more reliable results can be obtained on the relationship of populations by working with known major genes, such as those associated with the serological characters. If our primary interest is in the problem of the relationship of 2 or more human populations, then we ought to be interested in known major genes, and not in genetically unanalyzed traits like stature or facial form. If our primary interest is comparative human morphology, then of course, we ought to study stature and other normal morphological variations.

DAVID: The fact that it is difficult to analyze single-gene inheritance does not necessarily mean that an attack, at a different level, on polygenically determined variation would involve multiplication of the difficulty. We can predict, for example, the force of the exploding fuel in the cylinder of a gas engine, and the direction of its action, with infinitely greater ease than we could achieve the probably impossible task of ascertaining the individual energies and respective pathways of all of its component molecules.

DR. WILLIAM C. BOYD (*Boston University*): I do not feel so pessimistic about the possibility of genetic analysis of human morphological traits. The blood group differences without exception have proved to be analyzable in terms of the action of a relatively small number of genes acting in typical Mendelian

fashion. I think the number of genes involved in morphological differences may not prove unmanageable, when analysis is finally well started. Take the question of physical appearance, the resemblance between relatives such as father and son or brother and sister. Often it is very close, so close as to be startling. If appearance depended upon the action of many genes, each having but a small effect, this sort of "spitting image" resemblance would surely be less common. Polygenes may be involved, but I predict that a large part of the inheritance of morphological traits will prove to be due to relatively small numbers of genes acting in fairly simple manner.

Ascertainment and the Study of Discontinuous Characteristics in Man[1]

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IN GENERAL, genetic studies of discontinuous characteristics are initiated to answer any one or some combination of four basic questions, namely, to determine the mode of inheritance of a specific characteristic, or if this is known, the linkage relationships of this with other characteristics, the selective advantages or disadvantages of the character-bearer, or lastly, the frequency with which the trait arises spontaneously, presumably due to mutation. To this end families are selected for study. The biases introduced into the data through the method by which such families come to the attention of, and are recorded by, the investigator forms the core of the most pervasive problem facing the human geneticist, the problem of ascertainment. It is to the general topic of delineating these biases that I should like to address myself. It is important, however, that at the outset we distinguish between a consideration of the means by which data are collected and the method of analysis of such data. We shall concern ourselves primarily with a consideration of the former on the thesis that when the biases are fully specifiable a unique statistical test exists, unique in the sense of a most efficient test.

Families may be selected or ascertained for genetic study in either of two manners. Firstly, we may select families at random without reference to the type of offspring produced. When this obtains and if selection is truly at random, that is, if each family has equal probability of being selected for study, no bias is introduced into the data. Unfortunately, random selection is of limited utility in human genetics. All too frequently we are interested in a rare inherited trait where random selection will necessarily lead to a preponderance of families which could yield no information since the majority of families so selected would not contain individuals possessing the attribute in question. Moreover, the majority of randomly selected families would be incapable of producing an individual of the specified type. Consequently, in random selection we are interested not in a random sample of all families but rather in a random sample of families from a population of families of a specific mating type. It is feasible to delimit this population only when the mating type is recognizable because of the parental phenotypes. This would, in general, only be true in the case of dominantly inherited characteristics. More frequently

[1] Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1954.

we are faced with the situation where the parental phenotypes alone afford no clue as to the type of mating as, for example, in the case of rare recessively inherited pathologies. When this is the situation we are generally led to the second type of selection, a selection of families which contain at least one individual exhibiting the trait of interest, the affected person serving to identify the mating. This type of selection is termed "ascertainment through individuals" in contradistinction to the previously mentioned type of selection which is termed "random ascertainment of families." It has been customary to think of ascertainment of families through individuals as occurring only through affected persons, and that a family may be ascertained as many times as there are affected persons. These repeated ascertainments of the same family are said to be independent if the probability of ascertaining any one affected member of the family is stochastically independent of the probability of ascertaining any other affected member of the family.

When ascertainment is through individuals the exhaustiveness of one's search can materially influence the results. If the search is exhaustive, that is, if each and every affected person in a population is independently ascertained, ascertainment is said to be complete. In this instance the only bias introduced into the data is that due to the omission of those matings which, while potentially capable of producing individuals of the type being studied, fail to do so. In this case the analysis of the data is straightforward with the test of preference being the maximum likelihood test proposed by Haldane (1932). In practice, however, the most common situation is not one of complete ascertainment but rather one in which we fail to ascertain independently all of the affected individuals in the population; that is to say, in any given study ascertainment is most frequently incomplete. Under these circumstances if the level of ascertainment is sufficiently high, say 90 per cent or more, the error introduced by assuming complete ascertainment when analyzing the data is generally negligible provided the probability of ascertaining an affected individual is reasonably constant throughout the population. Unfortunately, ascertainments as high as 90 per cent are the exceptions rather than the rule in medical genetics. If ascertainment falls below this arbitrary level, as it must in poorly defined but relatively common clinical entities, the assumption of complete ascertainment is wholly unjustified and is apt to lead to gross inaccuracies in the estimation of the segregation ratios. Appropriate methods of analysis do exist for some of the situations which may arise when ascertainment is not exhaustive. Three such are the cases of (1) single selection, that is, no family is ascertained more than once, (2) multiple incomplete ascertainment when the number of propositi is unknown but an estimate of the probability of ascertaining an affected individual is available, and (3) multiple incomplete ascertainment when the number of propositi is known and no estimate of the probability of ascertainment is available. The analytical methods appropriate for

these three cases are due to Weinberg (1912), Fisher (1934), Haldane (1938), and Bailey (1951). These tests all presuppose a constant probability of ascertainment, and, obviously, a monomeric form of inheritance constant throughout the sample.

The difficulties which may arise due to the improper specification of the level of ascertainment have been excellently demonstrated by Fisher (1936) in connection with Haldane's work on partial sex-linkage. In reviewing Haldane's data under different assumptions with regard to ascertainment, Fisher finds that not only do the estimates of the recombination frequencies vary but the actual gene order is changed. The effect of differing assumptions regarding the level of ascertainment on the estimation of segregation ratios may be illustrated with the use of Sjögren's (1943) data on the recessive form of Friedreich's ataxia. Sjögren studied 100 cases of this disease distributed among 310 children of 63 families. Family size varied from one to 12 offspring. In these data, if complete ascertainment is assumed, we estimate the proportion of affected individuals from the mating of two heterozygotes as 22.8 $\pm$ 2.95 per cent, a finding compatible with simple recessive inheritance. Under the assumption of 80 per cent ascertainment, we estimate this proportion to be 21.1 $\pm$ 3.9 per cent. This, too, is compatible with simple recessive heredity. Lastly, under the assumption of single selection, we estimate the value of this proportion to be 15.0 $\pm$ 2.27 per cent. The effects of ascertainment on our interpretation of the data are obvious here.

Thus far we have concerned ourselves with a review of the conventional approach to the problem of ascertainment as developed largely by our British and German colleagues. As Sir Ronald Fisher (1936) has pointed out, the conventional approach has a number of deficiencies, the primary one being that writers on the theory of ascertainment have not had a familiarity with the selective process through which family histories and patients necessarily pass before they are put on record. These criticisms, while made almost 20 years ago, are still valid. I should like, therefore, to explore in some detail the selective processes in a large-scale study of an inherited anomaly of man.

Somewhat over two years ago, we at the Heredity Clinic launched a study of multiple neurofibromatosis or von Recklinghausen's disease. The principal objectives of this study were to provide additional clinical and genetic data on the manifestations of this disease, to determine the selective factors tending to limit its distribution, and to estimate the frequency with which neurofibromatosis arises as a consequence of mutation. It was apparent from the outset that random selection of families was not practicable because of the uncommonness of neurofibromatosis. Ascertainment, therefore, could only be through affected individuals. Available to us were 134 index cases or propositi distributed among 127 families. These individuals had come to our attention from four sources. The first source was the records of the University of Michigan Hospital for the

period 1934–1950. In this interval the diagnosis of neurofibromatosis had been made on 228 individuals. But, for a variety of reasons, in only 87 cases could the patient and his or her family be adequately studied. Of the 141 cases which could not be included, 20 are known to be misdiagnoses or instances of patients with solitary neurofibromas arising in a region previously traumatized. Of the remaining 121 cases, 48 per cent were discarded because of failure to locate the patient or his family or because of non-cooperation. We have no idea as to how representative or non-representative these 121 cases may be. To analyze the families which were studied we must assume that the discarded cases do not differ from the selected ones in the frequency of sporadic cases, of different variants of the disease, etc. How valid this assumption may be is a matter of speculation.

The second source of propositi was the University Hospital admittances during the period 1951–1953, that is, after the initiation of our own study. Thirty-one individuals came to our attention in this manner; 26 of these individuals were incorporated into the study and the others discarded because of solitary tumors or misdiagnoses. From the standpoint of ascertainment the distinction between this and the preceding group is clear. Only in the latter cases did we see the propositus at his or her initial admission. The third source, from which 17 cases were derived, was a survey of four state mental institutions. While these cases were useful in the estimation of the frequency of neurofibromatosis, the family histories which were obtained were too poor to permit the addition of this group to the others for the more genetic aspects of the study. The fourth and final source was a series of miscellaneous ascertainments including cases referred to us directly by outside physicians, adoptive placement problems, or interested laymen, "snoopy neighbors" if you will! This miscellaneous group excellently illustrates that the conventional dichotomy of ascertainments into random selection of families and selection through affected individuals is not exhaustive. In the adoptive placement problem ascertainment was through a normal individual.

The 127 families were contacted by field investigators and appointments made for examination of the available members of the family by a staff dermatologist. Upon completion of the family record the 134 propositi were classified as "sporadic" or "familial." The use of the term sporadic here is not quite the conventional one. The terms sporadic and familial are used with reference to the propositus alone. The propositus is defined as a sporadic case if neither of his parents or none of his siblings had neurofibromatosis. At this stage in our thinking we naturally became interested in a comparison of the factors leading to the ascertainment of individuals in these two groups. To the end of making such a comparison, we classified the propositi on the basis of their chief complaint at the time of the admission at which neurofibromatosis was a clinical finding. We recognized four general types of complaints, namely, complaints

which were (1) not related to neurofibromatosis, (2) related to neurofibromatosis but where the patient did not realize that this was the cause of his complaint, (3) related to neurofibromatosis and the patient was aware of this, and (4) on a purely cosmetic basis. This scheme of classification revealed interesting differences between the two groups. Approximately the same percentages of familial and sporadic cases were admitted with complaints not referable to their neurofibromatosis, or with complaints directly related to their disease and of which they were aware. However, 46 per cent of familial cases as opposed to 30 per cent of the sporadic cases came to our attention through complaints referable to their neurofibromatosis but not realized to be such at the time, such as rotoscoliosis, bony nonunions, etc. On the other hand, 26 per cent of sporadics as opposed to only 10 per cent of familials were admitted with complaints which were essentially cosmetic in origin. In other words, the sporadic case was far more frequently concerned about the unsightliness of his disease than the familial case. This finding led us to an attempt to appraise the representativeness of the propositi for the familial cases. In 13 families it was possible to compare the propositus with affected siblings who were either older than or at least as old, at the time of our examination, as the propositus at his admission to the hospital. In 11 of these families the propositus was more severely affected than his affected siblings. Thus in only two families were the propositi representative of affected individuals in their sibships. This in turn militates against the assumption of a constant probability of ascertainment, an assumption inherent in the conventional approach to the problem of ascertainment.

Let us turn now to another problem in ascertainment posed by these 134 cases of neurofibromatosis. We have mentioned that they are distributed among 127 families; this obviously means that in some instances two or more members of a family were admitted to the hospital during the period of this study. To be exact, we have 7 families in each of which two persons were ascertained. Do these 7 cases constitute multiple independent ascertainments of these families? Unfortunately, independence, while readily defined, is not so easily determined in the individual case. As Bailey (1951) has pointed out, rarely can multiple ascertainments be considered independent when the individuals concerned are visiting a hospital for treatment. In 4 of our 7 cases, one of the propositi underwent surgery at the University Hospital. These cases, then, must be suspect. In the remaining 3 cases, only one case can legitimately be assumed to be an instance of double ascertainment. In this case one member was ascertained through the University Hospital and the other through the survey of mental institutions.

There remains but to consider one more problem which while possibly not directly a problem in ascertainment is certainly closely akin to it, and is what may be called the "hierarchy of reliability." This hierarchy may be illustrated

by the neurofibromatosis data in two different ways. Firstly, with respect to the individual, either affected or normal, we may have information obtained from several sources. To name but a few: we may have information from the propositus and/or other members of the family, from a trained field worker, from a local physician, from an outside hospital, from death records, or from the physician in charge of the clinical aspects of the study. Neurofibromatosis in some ways presents a unique opportunity to evaluate crudely these different sources of information. Least reliable on the average are the reports of the propositi and their affected relatives. This is in no small measure due to the mental retardation frequently associated with the disease. Death records we have found to be unreliable. We have higher regard for information obtained from outside hospitals and local physicians. The next order of reliability would be our field workers who, while not medically trained, are observant and have seen far more cases of neurofibromatosis than the average physician. Lastly, our greatest confidence is placed in the clinical specialist. While this crude ranking may not receive your acceptance, I'm sure that agreement will be reached on differences in reliability.

How are these differences in reliability to be allowed for? The answer is by no means obvious. On the one extreme, we could ignore them and treat all observations as having equal merit. This hardly seems justified. On the other extreme, we could accept only those observations made by the clinical specialist. This would lead to sacrificing information which is both valid and germaine.

The second illustration of the hierarchy of reliability arises in the following manner: to estimate mutation frequency by the direct approach we must determine the proportion of sporadic cases among all cases of neurofibromatosis. To certify that a given case is sporadic requires that both parents be examined. On the other hand, a familial case becomes certain on the examination of only one parent if that parent happens to be the affected one. To require then that for acceptance of sporadic cases both parents must be examined is to place a more rigorous definition on one group than on the other. In our own study, 74 individuals are "sporadic." Of these 74 cases, in 30 instances both parents were examined, in 14 only one parent was examined, in 10 neither parent was examined but some siblings of the propositus were, and in 20 no other member of the family was examined and we have only the family history to suggest that the case is sporadic. How are we to weight for these different levels of reliability? To discard any which were in fact sporadic cases, whether or not examinations were conducted, could only lead to underestimating the proportion of sporadic cases and ultimately the frequency of mutation. Here possibly the biometricians will have an answer.

In summary, the purely practical aspects of ascertainment and reliability of information require more careful description and delineation by the human geneticist before precise analytical tools can be developed. A few of our own

feelings in this regard have been presented for your consideration. It is to be hoped that from this meeting through our combined experiences in the collection of genetic data a beginning can be made on this description.

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Some Problems in the Study of Quantitative Inheritance in Man[1]

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THE hypothesis that the genetic part of quantitative variability depends in general on multiple gene loci distributed over the chromosomes is the most reliable explanation of the observed characteristics of continuous variation in man and other organisms. The hypothesis is not entirely satisfactory because it cannot adequately be tested (Haldane, 1946, Hogben, 1951, Wright, 1952).

In the formal genetics of discontinuous variations, hypotheses on mode of inheritance can be formulated and tested with accuracy. Discrete characters are associated with a specified number of genes according to rules reflecting the patterned and random regularities of chromosomal behavior in gamete and zygote formation.

We assume that continuous variation represents the combined effect of multiple gene and environmental determiners. Compared to the case for dis-

[1] Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

continuous variations, this assumption is vague. Because of the vagueness it is extremely difficult to formulate testable hypotheses regarding the detailed mode of inheritance for continuous variations. The chief difficulty is the large number of contributing variables. Since we cannot formulate a precise hypothesis, it follows we cannot predict test results within close limits. Further, the lack of a precise and appropriately detailed model means that any estimate based on the imprecise model will not be efficient (Nedler, 1953).

Thus, for the present, if we are to investigate continuous variation in man, we must use inefficient methods of analysis. The prospect is not good that we will soon know enough to have a predictive understanding of continuous variation in human individuals. However, the methods we have are superior to the published materials for the study of quantitative genetics of man. These methods will be considered by the discussants: Drs. Lush, Comstock, and Dempster. My assignment from Dr. Green is: "First, to present some actual data on quantitative traits in the Navaho Indians, and Second, to confine the presentation to thirty minutes." I am instructed: ". . . merely to allude to methods of analysis while pointing out questions of genetic interest. Thereby it will be possible to depend upon the discussants to present methods and evaluation of the methods."

The complexity of the genetics of quantitative variation is illustrated by fig. 1 (adopted with major modifications from Lerner, 1950). What we observe is phenotypic variation. After analysis, phenotypic variations may be divided into two sorts: 1) characters, and 2) traits. Here "characters" mean phenotypic attributes or attribute sets whose variation (for a defined environment) has been demonstrably associated with a defined set of genes. The definition of "character" presupposes certain specific genetic information. Since there are a limited number of genes in man, there are a limited number of characters. Generalizing from known cases the variation of characters is usually (but, theoretically, not necessarily) discontinuous. "Trait", as used here, means all phenotypic attributes or attribute sets that are not "characters." No specific genetic information (or, negative information alone) is presupposed in the definition of traits. The variation of traits is often (but not necessarily) continuous. Traits may be associated with "factors", that is, with unidentified genes. Statements about factors (as in the sentence "stature is controlled by multiple factors") presuppose different prior information than statements about genes. By genetic analysis with positive results traits may become characters. Some such distinction between characters and traits, between genes and factors, helps to keep exposed our ignorance of the genetics of many human variations. The Navaho variations to be discussed in this paper are traits. Before considering these traits, however, we need to take a quick view of some background information on the genetics of human populations.

In theory, the variation of characters may be proportioned into two sources:

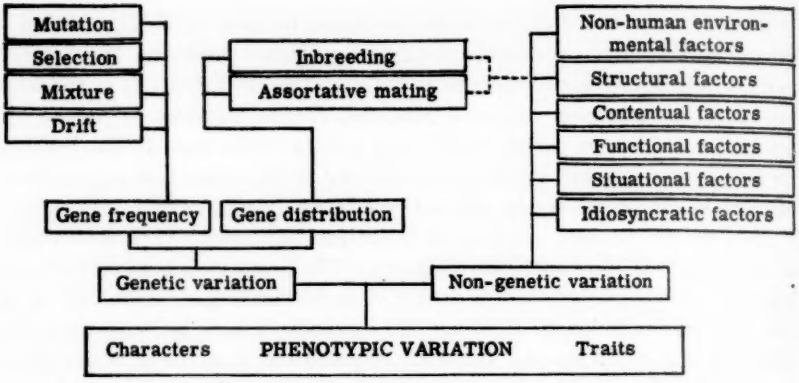

FIG. 1. A scheme showing some determinants of phenotypic variation

1) Genetic variation, and 2) Non-genetic variation. The genetic variation of a population is a function of the frequency and distribution of genes. The major modes of change of gene frequencies are mutation, selection, mixture between breeding populations, and genetic drift. The conditions of gene distribution within a breeding population are set by the system of mating. It is not necessary, before this audience, to characterize the modes of change nor the requirements for a steady state of population gene frequencies and distributions. Later, examples will be given to show how knowledge about inbreeding and assortative mating may aid the study of quantitative inheritance.

The modes of change and the conditions of steadiness for the non-genetic fraction of human variations are the subject matter of a wide variety of physical, life and behavior sciences. The scheme for the non-genetic part of fig. 1 is an adaptation of one suggested by Kluckhohn and Mowrer (1944). If we start with a given item of individual behavior in a societal context we may analyse the background of the behavior by abstracting out those factors which are resident in persons or groups of persons (structural, contentual, functional, idiosyncratic and situational factors) as opposed to those that are not resident in persons (non-human environmental factors—geographical, non-human organismal factors, etc.). Structural factors refer to the ways individuals get status positions in society. Sample names of status positions are "father," "mother", "citizen", "geneticist". Contentual factors refer to the ways individuals get roles for acting in a given status. The behavior in two societies with the same structure may differ because of variation in the roles assigned to individuals in similiar status positions. Individuals occupy a variety of statuses and roles simultaneously. The behavior in one status-role often is not independent of behavior in another status-role. Functional factors refer to the interconnectedness of these in ongoing behavior. Those aspects of individual behavior not referable to structural, contentual, functional or situational factors

are called idiosyncratic factors. Finally, the behavior of two individuals within the same structural, contentual, and functional framework may differ, to an extent greater than can be assigned to idiosyncratic factors, because of variation in the situational factors—the precipitate of past experiences, and all other things which define the situation in which particular behavior occurs.

This is not the place to discuss in detail methods of analysis of the non-genetic component of human quantitative variability. The difficulties of a satisfactory analysis here are probably greater than for the genetic component. Satisfactory resolution of problems of quantitative inheritance will require better methods of analysis of the non-genetic component. Geneticists could make much fuller use of the methods developed by the behavior and life sciences for analysis of human environments.

There is no single scheme for the interpretation of human behavior which is both a) widely accepted among behavior and social scientists, and b) adequately verified by empirical testing. Although some such scheme is known to be necessary for studies of the quantitative genetics of human behavior, most of the ways in which such studies may be valuable in the study of morphological and other non-behavioral variations have yet to be explored. There is time to sketch a single example.

Consider the system of mating. The distribution of genotypes in a population is a function both of the kinds and frequencies of genes present and of the system of mating. No human society is known where mating is random for all possible criteria (excluding sex). But the cultural criteria for mate selection vary greatly between societies. For instance, with regard to inbreeding, in some societies brother-sister mating is socially sanctioned (Ptolemaic Egypt), or at least acceptable (the Caiçara of Brazil, see below), while in others it is taboo. With regard to assortative mating, in some societies (to pick a social criterion) a progeny-tested unmarried female may have high, and in others, distinctively low marriagibility. Structural factors determine what kinds of relatives—biological and social—are recognized. Contentual factors and functional factors set the rules for behavioral interaction of these relatives. Within the area of behavior which is permissive rather than required, observed instances of behavior can be explained further by idiosyncratic and situational factors. In many societies a male's mother's brother's daughter is an ideal mate. But whether two particular cross-cousins do in fact marry (and thus conform to approved but not required behavior) is a consequence of the idiosyncracies and the past experiences of the two individuals. Within the limits set by demographic factors—themselves determined by an interplay of biological, social and cultural factors—the system of mating in human populations has socio-cultural determinants; and, in turn, the system of mating is a codeterminant of the distribution of genotypes and thereby of quantitative morphological variations in the population.

Before I talk about the Navaho, I want to make, but not to elaborate here, one more methodological observation. It is simply this. Not only do we need to have bigger and better methods of analysis from the statistical side, but also we need to find and to use more suitable material for our investigations. I do not mean organisms other than man. To do this we will have to study subjects other than school children and other individuals who can be comparatively easily ascertained because they can be controlled through an institutional setup. Two items tell the sort of thing I have in mind: 1) Human inbred material does occur in highly useful amounts and places. Yet inbred human material has not been used in recent studies on quantitative genetics. 2) Fertile multiple mating occurs in almost all human societies (cf. our own serial polygyny). Yet nothing comparable to the between-sire and between-dam methods of animal genetics has been used in human studies.

NAVAHO DATA[2]

Two traits will be used to illustrate some problems met in the study of quantitative variation in human populations. The data are from the Ramah Navaho, a local group of Indians living in west central New Mexico who numbered 614 individuals in September, 1950. The present population was founded in 1868 by individuals born about 1820–1840 and is now in its eighth generation. The genealogy of about 1200 members, living and dead, is known.

The two traits are stature and head height. Stature was selected for discussion, first, because it is the most extensively reported quantitative human trait, and, second, because, from the time of Galton, it has been the most popular quantitative trait for human genetical analysis. The second trait, head height, was selected because it has diagnostic value in distinguishing regional American Indian morphological varieties, for example, the eight varieties defined by Neumann (1942, 1952) for aboriginal North America, and because it illustrates certain difficulties of interpretation for quantitative traits.

The distribution of these two traits for five subgroups of the Ramah population will be given. The five subgroups are:

1. Total adult series
2. Adult offspring of related parents, that is, inbred adults
3. Adult offspring of unrelated parents
4. Unrelated parents
5. Related parents

Stature was measured with an anthropometer following standard technique. Measurements were read to the nearest millimeter. In the males the range of values is 150–183 cm., the mean is 167.32 ± 0.53, and the standard error is

[2] Ten tables of Navaho data, presented as slides at the conference, have been omitted from this paper.

5.31 ± 0.05. In the females the range is 145–165 cm., the mean is 156.42 ± 0.55, and the standard error is 6.03 ± 0.05. These values were obtained before rounding to centimeters. The values for the Total Adult Series are the values for the 221 individuals measured.

You will note immediately that the sample shown here (103 males and 118 females) is smaller than 614, the size given earlier for the Ramah population in 1950. This illustrates one serious problem of genetic studies in human populations—the samples are never ideally large. Of course, usually this is a practical rather than a necessary difficulty. Adequate studies on human subjects are very expensive both in money and time, especially where the subjects represent a "natural" population as opposed to an institutionalized group.

Actually our total measured Ramah Navaho series is 482 individuals. It is a judgement sample selected to be representative of the group with regard to geographical location of family homes, sex, age, degree of acculturation, economic and social status. The sample constituted 78.5 per cent of the total population.

Here we have reduced our sample size by 45.8 per cent by excluding observed children, that is, persons under 17 years of age. We could include the children in a study of quantitative inheritance by employing one of a number of procedures for correcting measurements of children to predicted adult values. While such corrections are never fully satisfactory (different children move through a generalized growth space on different paths and at different rates), the careful use of age corrections permits fuller utilization of information for the parent-child relationship. If ample material is available the analysis can be kept more simple by excluding the children. However, the growth cycle difficulty is not entirely removed by limiting the analysis to adults—the ontogenetic cycle in man is such that parents are usually somewhat worn, shrunken, bent or broken by the time their children reach adulthood. Usually there is not sufficient material to restrict the data to a narrow age level.

Head height was measured in millimeters following the Harvard technique with the anthropometer. In the total series the males have a range of 114–139 mm., with a mean of 126.80 ± 0.56 and a standard error of 5.62 ± 0.06. The females have a range of 111–136 mm., with a mean of 124.33 ± 0.51 and a standard error of 5.53 ± 0.05.

Before looking at the correlation for stature and head height among the various subgroups, let us consider briefly an important point suggested by head height. The problem of what to measure and what is measured is a persistent difficulty in the study of quantitative genetics in man.[3] Often measure-

[3] There is not time to discuss in any detail the problems of observational error and of scale. On the problem of scale see, for morphological traits, Mather (1949) and Wright (1952), and for behavioral traits, Stouffer, et al. (1950). On observational errors in physical anthropometry see Boyd (1929) and Davenport, Steggerda and Drager (1934).

ments can be made quite accurately between two or more anatomical points. But the biological stuff between the points may be quite heterogeneous between individuals (Washburn, 1951).

On the living, head height is measured from porion to vertex. On the skull, or on a lateral x-ray plate, the height measurement is usually the basion-bregma diameter. The range of Navaho head height measurements is from 111 to 139 mm. Conventionally these measurements are classified into three categories: high, medium and low head height. Suppose we have a head height measurement that falls in the category "low head height." There are at least four possible interpretations of this datum:

1. The entire skull may be small, and thus the head height not relatively low at all,
2. The skull may in fact have a relatively high vault but the base may be flat—the declination of the pars basilaris on the Frankfort plane is such that the lower component of the height is short,
3. The skull may have a relatively low vault—one with the upper component of the height short, or
4. The skull may show a combination of low vault and flat base.

A number of workers, mainly anthropologists and osteologists, have suggested that if we subdivide the traditional measurements such as head height into their morphological components, we would find that the variation of the individual components would follow more simple modes of inheritance than that of the "total" measurement. I doubt very much that this will prove to be a general finding, but I do feel that redefinition of the "parts" of the human organism—as in morphological component analysis—will help us to arrive at more consistent results, especially for some kinds of anthropological problems.

We are now ready to look at the correlations for the distribution of these traits in pairs of relatives. For present purposes, the comparisons are restricted to same-sex pairs. As in all Primates phylogenetically later than the gibbons, there is considerably sex dimorphism in the Navaho. For instance the stature of Navaho females in this sample of adults ranges over 20 units on the centimeter scale, while the range for males is 33 units, the mean differences between the sexes being 10.9 units. In actual analysis traits with different distribution for the sexes, of course, should be handled separately unless the values for one or both sexes are transformed. The argument is more simple when we restrict analysis to the same sex but the restriction reduces the amount of information available on a natural population by at least one half. The alternatives to such a restriction include that of expressing the values of one sex in terms of the other, and of using the notions of mid-parent and mid-offspring.

Restriction of comparisons to the same sex is accompanied by another difficulty. Given that genetic factors are involved in the variability of the trait under consideration, then to the extent that positive phenotypic assortative

mating occurs, the child will resemble the like-sexed parent not only because of factors received from that parent, but also because of factors received from the unlike-sexed parent, the one not entered into the same-sex correlation. The correlation within 77 Navaho mated pairs is $r = -0.18$ for stature and $r = +0.08$ for head height. The 95 per cent fiducial limits are $-0.55 \leqslant r \leqslant +0.25$ for stature and $-0.42 \leqslant r \leqslant +0.48$ for head height. The data given here are not sufficient to tell us that assortative mating safely can be ignored in any analysis of the genetics of Navaho stature and head height.

It is of incidental interest to suggest that knowledge of the breeding structure of a population may be essential to a proper understanding even of traditional anthropological results where populations are described in terms of normal curve statistics. Gini (1950) has shown that the distribution of stature in some large and seemingly representative European population samples is leptokurtic. W. Lenz (1952) suggested that this finding would be expected under a system of positive phenotypic assortative mate selection for stature, and, further, that a number of European groups had been shown to practice this kind of mate selection. But some populations with platykurtic distributions are known. In general it would seem that a quantitative genetic trait would be expected to show a leptokurtic distribution under a system of positive phenotypic assortative mating and a platykurtic distribution under a system of negative phenotypic assortative mating.

Parents are classified as unrelated when they have no biological relationship in the eight generations known for the Ramah group. Related parents, of course, have one or more common biological ancestors within the known parental generations. The population mean coefficient of inbreeding over all eight generations (1118 individuals) is 0.0066 (Spuhler and Kluckhohn, 1953). The mean coefficient for inbred sibships, over the last four generations, is 0.0175. The highest coefficient for an individual sibship is 0.0977 (where the parental, multiple relationship is full first cousins, full first cousins once removed, and full third cousins), and the lowest coefficient for an inbred sibship is .0005 (half fourth cousins).

The Navaho inbred material reported here is by no means the best that could be made available. Although the mean inbreeding coefficient is about four times the estimates reported for Europe and two times those reported for urban areas in Japan, it is lower than the estimate (.0254) for the Dunker isolate in this country (Glass, et al., 1952). In terms of population size the amount of inbreeding at Ramah is relatively small. The operation of cultural values keep the Ramah coefficient low. The point that needs to be made is that populations are available where considerable inbreeding is in practice. For instance, Willems (1952) has reported (from the standpoint of cultural anthropology) some Caiçara communities on the southern coast of Brazil where both father-daughter and brother-sister matings produce offspring with some frequency,

and this with local social approval. Study of even a small number of such cases would be of great value. Further, Ellis (1936, p. 142), in a paper based on the genealogical studies by Silva Leme (*Genealogia Paulista*), found 42.8 per cent of marriages were consanguine among the upper classes of the Plateau of São Paulo during the 19th century, and the degree of inbreeding in this stratum of the Planalto Paulista population appears to be increasing rather than decreasing. Ethnological information on numerous other inbred populations can be found in the anthropological literature (Lévi-Strauss, 1949; Murdock, 1949). But as is shown by the work of Eaton, Glass, and Steinberg on isolates in this country, you need not leave home to find suitable material for study of the quantitative genetics of man.

Data will not be presented here on the few known sets of Navaho twins. Dr. Kallmann will discuss twin methods. The great advantage of twin methods for the study of quantitative genetics in man (assuming there are only two sorts of twins and the methods for diagnosis of the two sorts are trustworthy in practice) is that it should measure all of the epistatic and dominance variations as well as the additive ones. A disadvantage is that for most practical problems, we want to have information relevant to singletons rather than to twins who never constitute more than a small fraction of natural human populations. There have been less than a dozen pairs of twins in the history of the Ramah population. Also, there seem to be some primary difficulties in transferring statements about quantitative inheritance in twins into statements about singletons (for examples of such difficulties see Price, 1950).

SUMMARY

Some problems in the study of the quantitative genetics of man are discussed using data from the Ramah Navaho Indians of New Mexico as illustrative material. Information is presented, but not analyzed, on the distribution of stature and head height in the adult Ramah Navaho sample for parent-child and sib-sib pairs, for related and not related parents, and for inbred and not inbred offspring.

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DISCUSSION

Moderator: Dr. Earl Green, Atomic Energy Commission

GREEN: The discussants for Dr. Schull's paper on "Discontinuous Traits" are Dr. Steinberg, Dr. David and Dr. Kemp.

STEINBERG: Much of what we have heard presented has been about the severe difficulties that confront human geneticists. I tend to feel that the difficulties, in many instances, are not as great as they are stated to be. My own experience has been limited exclusively to working on the determination of the mode of inheritance of certain characteristics. Only recently have I begun to worry about problems of linkage—and a very little about mutation rates. My very brief comments will be weighted accordingly.

With regard to ascertainment of families for determining modes of inheritance of diseases, Dr. Schull has emphasized that the classical techniques make the assumption that the probability of ascertaining each individual bearing the disease is equal. I would like to submit that that is not a necessary assumption in these hypotheses. If there is no correlation of the degree of manifestation of a disease among the members of a family, the probability of ascertaining families of a given size with equal frequency of manifestation of the disease is, on the average, equal. If this condition is fulfilled, it seems to me that the requirements for the classical methods of calculating the mode of inheritance are fulfilled. Hence, equal likelihood for ascertaining each affected individual is not necessary, but lack of correlation of the degree of manifestation of a disease among the members of families is necessary. On the other hand, if there is considerable correlation of the degree of manifestation of the disease among members of a family, I think it is pertinent to seek out possible basic differences in the disease among the several pedigrees and to seek modes of inheritance for what may be a group of similar but basically different diseases. This sort of problem arose in the study of symphalangism. It was claimed that all symphalangism was due to one dominant gene. However, when you examine pedigrees, you find, without exception, that the same joint is affected in all members of the family showing the character. It may be either the proximal or the distal joint or both joints within a pedigree. However, if it is the proximal joint that is affected, no member of the pedigree will have the distal joint affected. The number of proximal joints affected may vary, but the variation is never from proximal to distal joints. If it is the distal, then no member will have the proximal joint affected, and, finally, if it is both joints, all affected members will have both joints affected. Because of this, it seems reasonable to assume that not all symphalangism is due to the same mutant gene.

The same sort of problem turned up with regard to sickling and the sickle cell anemias where again by more careful techniques it was possible to show there were other mutations involved.

With regard to the 13 families with more than one proband, my initial remark applies. Dr. Schull stated that in eleven of the cases the probands were more severely affected than the affected sibs. The remark was made that this mitigates against the assumption of equal ascertainment. It does for individuals, but not for families and therefore does not invalidate the use of the standard techniques of data analysis.

With respect to mutation rates, I agree that variable expression makes their determination difficult. As I have said, I have just begun to think about the question of linkage. The problem is best approached by using as one of the loci something like a blood group factor which is not selected for study in the usual way.

Finally, I think we can all agree that using data from the literature in which

the method of obtaining the cases has not been described is at best only an indication of what sort of data should be collected in the future. To draw conclusions from such data is foolhardy indeed.

DAVID: I am glad that Dr. Schull has seen fit to discuss the question of ascertainment because it seems to me that although papers dealing with this problem have been available in the literature for some time, we still find reports coming out in which the problem is altogether ignored. As Dr. Schull points out, the analytic results you get in dealing with data which have been ascertained in one way on the assumption they have been ascertained in another can be rather badly distorted.

I don't want to quibble about terminology, since any terminology would probably have at least as many objections as that used by Dr. Schull, but I wonder if in place of referring to "random selection in families" vs. "ascertainment through individuals," it might not be as simple, and perhaps more explicit, to use "indirect ascertainment," as contrasted with "direct ascertainment," the indirect ascertainment of cases referring to the situation in which you survey families without regard to prior knowledge that they may contain affected individuals and thus find affected individuals indirectly, and direct ascertainment relating to the situation in which you search directly for affected individuals. In both cases, of course, the families are ascertained through the affected individuals they contain. On the other hand, a sample composed of the children of sisters of hemophilics, for example, would not be ascertained through its affected members, and would require still different analytic treatment.

Now Dr. Schull raised the problem of what to do when the rate of ascertainment (i.e., the probability of individual ascertainment) is not discernible from your material, even if you are willing to assume that the rate is more or less constant from one family to another; what to do when you cannot estimate the ascertainment rate from your data, and in fact when you cannot specify in any precise way just how the material was ascertained. Let us say that the sample has been obtained in some such irregular fashion as was illustrated in certain of Dr. Schull's examples. I think the only thing you can do then is what Haldane proposed in his 1938 paper. That is, make estimates of the familial incidence based both on the limiting assumption of exhaustive ascertainment on the one hand and on that of single ascertainment on the other. These estimates, with appropriate consideration of their standard errors, provide a sort of confidence interval within which the true familial incidence may safely be presumed to lie. This procedure is probably applicable to almost any collection of data in which there has been no arbitrary rejection of cases simply because there were not enough of them to a family to make them "interesting." It is probably not validly applicable, however, to just such material as Haldane

chose to use for illustrative purposes, that is, material assembled from occasional literature reports, as contrasted with that derived from systematic surveys.

The difficulty in analysis which is imposed by uncertainties respecting method and rate of ascertainment obviously emphasizes the desirability, where it is feasible, of setting up an investigation in such a way that you may establish in advance the actual rate of ascertainment. In many or most practical situations this is not possible. It would not be possible in most of the situations with which Dr. Schull and his associates are concerned, where they must depend on hospital referrals. But if you were studying the familial incidence of a moderately common trait, one with a population frequency in the neighborhood of two or three per cent, you might be in a position to set up your ascertainment procedure in such a way that the ascertainment rate would be established in advance. You might have the choice of proceeding in a search for affected individuals vertically through a series of diverse age groups—by going through an entire school system from the top grade of high school to the kindergarten, for example; this would permit the direct ascertainment of more than one sib, in families with several affected members, and you would wind up with some degree of multiple individual ascertainment. Alternatively, you might proceed horizontally, using a larger number of schools, but restricting your survey to a single grade or age group, which would result in single ascertainment or a close approximation of it.

"Exhaustive" or "complete" ascertainment implies that sibships of given size with different numbers of affected members are represented in the sample in the same relative proportions as in the general population; it entails an ascertainment rate of unity. Dr. Schull has noted that in many investigations in which there was multiple individual ascertainment an assumption that the ascertainment was exhaustive would be unwarranted. In fact, it is doubtful if a very close approximation to exhaustive ascertainment is ever obtained in practice. I suspect that Sjögren's data on juvenile amaurotic idiocy involve as high a rate of ascertainment as you will find, and there, if I remember rightly, the maximum likelihood estimate of the ascertainment rate is no higher than 0.7, with a substantial standard error, and the usual possibility of overestimation stemming from the assumption of independence in the individual ascertainments.

It is perhaps worth noting, because I am not sure whether or not it is common knowledge, that in general, single ascertainment may offer certain technical advantages over multiple individual ascertainment, whether the latter approaches exhaustiveness or not. Hence, if you are in the position, unhappily rather rare, of being able to choose your method of survey, a method designed to yield single ascertainment may be the preferable one. For one thing, with

single ascertainment, the analytic treatment of the results is greatly facilitated. This is conspicuously true for material in which age adjustments have to be made to allow for variability in age of onset. To my knowledge, no method has been devised which will yield familial-incidence estimates with assignable margins of error, when age adjustments have to be made, for data secured through multiple individual ascertainment. Even when age adjustment is not at issue, it appears that for equal effort, sample collection through single ascertainment may yield appreciably more information (on estimates of familial incidence) than that in which exhaustive ascertainment is approximated. In fact the amount of information per index case is about twice as great for single as for complete ascertainment.

DR. TAGE KEMP, M.D. (*University Institute of Human Genetics, Copenhagen*): In my discussion of the fine paper just read by Dr. Schull, I shall try to set forward some additional remarks on the basis of our practical observations in Copenhagen. The first item I would like to discuss is the problem of phenocopies. They have to be considered carefully as regards every characteristic or disease investigated. Congenital defects and diseases may be hereditary or acquired, and a non-hereditary lesion may be indistinguishable from a hereditary imitation. For instance even if most cases of harelip and cleft palate must be supposed to be hereditary, whereas among isolated cleft palate cases there is a considerable admixture of non-hereditary cases. This fact has to be considered, when a number of propositi with this malformation and their families have been collected and the material has to be analyzed to evaluate the mode of inheritance.

Item 2 has a certain relation to item 1. Item 2 concerns the importance of correct diagnosis. To obtain that we have in the Institute for Human Genetics in Copenhagen the following organization. We have in the Institute a Medico-Genetic Registry, including a systematic registration, a card index so far as possible complete, covering all the more serious hereditary affections observed in Denmark during recent years. In the Medico-Genetic Registry we have a starting point for more thorough studies of the inheritance of a special lesion or disease. The procedure then is as follows: The physician, who is trained as a specialist in the field concerned, makes a thorough investigation of the individual patients with the disease or the lesion in question and of their families, partly on the basis of hospital records, other documentary material and geneologic investigations, partly by travelling about, visiting and examining the individual patients in their homes or by calling the patients in to the Institute or to some hospital for observation and more thorough examination.

For this type of investigation a close and friendly cooperation with the medical profession, general practitioners, as well as doctors connected with

hospitals and institutions, and medical officers, is necessary. The medical students ought to have as part of their curriculum compulsory courses in general and medical genetics.

Item 3. A good way to obtain a correct ascertainment is to count all the cases of the disease in question in a country or in a certain district. I may mention the following examples of the procedure:

Recently Øster (1953) counted all the cases of mongolism on the Danish Island, Seeland, and neighboring islands, with a total of 1,800,000 inhabitants. He counted 526 mongols alive and examined them all and their families personally. He found the incidence of mongols among the new-born at 1:618 or 1.61 per thousand. The incidence in the population is one mongol per 3,978 individuals. He found another mongol in the sib-ship in 1.7% of the propositi and in 0.9% in the control material (the expected incidence of another mongol in the sibship). Thus, there was no significant familial accumulation nor any evidence to suggest that a woman who has once born a mongol has a greater chance of bearing another than other women of similar age. Among distant relations of the propositi 10 cases of mongolism were found (6,600 relatives). An accumulation beyond that which might be expected was not found.

Trier Mørch (1942) counted all the chondrodystrophic dwarfs in Denmark (86), and on the basis of material from the lying-in department he estimates the birth-rate for chondrodystrophic children to be 1:10,000. Only ⅕ survived the first year of life. The frequency of chondrodystrophy in the population is about 1:44,000. One mutant birth (*i.e.* a chondrodystrophic born of normal parents) in 12,000.

Andreassen (1943) counted 81 hemophiliacs living in Denmark as members of 63 families, comprising 1,970 members among whom 205 were hemophiliacs and 74 sure heterozygotes.

Birch-Jensen (1949) counted all the cases of congenital deformities of the upper extremities in Denmark and found the relative frequencies of the various types. He observed 15 per 100,000 in the population and 23 per 100,000 at birth.

Møllenbach (1947) found 40 persons with aniridia living in Denmark, in 1944. In 1940, the population amounted to 3,844,000. Thus the incidence of aniridia in the total population is taken to be 1:95,000. On the basis of 28 primary cases of aniridia that appeared between the years 1875 and 1944, the frequency of mutation for aniridia is estimated to be about 1:100,000, reckoning with a suitable margin of safety for cases that might not have been recorded.

Borberg (1951) investigated 37 cases of tuberous sclerosis (26 severe cases and 11 abortive cases). Twenty-two were alive. The incidence in the population is about 1.001 percent.

Strömgren (1938) took a census of the Danish Island, Bornholm (about

40,000 inhabitants) and Fremming (1951) carried out an investigation on a material of propositi consisting of the persons born on Bornholm within the five-year period, 1883–87, somewhat more than 5,500 persons. More than 92 percent of the propositi were traced and examined. On the basis of these investigations, the following frequencies were counted in the population: Feeblemindedness 1.3 percent (1 percent hereditary cases and 0.3 percent non-hereditary cases); 1.7 percent mentally retarded; psychopathy in 1.7 percent; schizophrenia in 0.3 percent (morbid risk 0.9 percent) and morbid risk for manic-depressive psychosis 1.6 percent (in males 1 percent, in females 2.2 percent).

These are instances of methods of ascertainment; general rules for ascertainment cannot be given; special methods have to be used for each type of investigation.

SCHULL: I have a number of comments, but first I should like to call attention to an assumption implicit though not explicitly stated, in the statements made by Drs. David, Kemp, Steinberg, and myself. This assumption which is all too frequently ignored in applying the present tests is that the trait under study is due to one and the same gene regardless of the family from which the affected individual is drawn. Now, with reference to the point raised by Dr. Steinberg regarding the probability of ascertainment. It seems to me that the assumption regarding the probability of ascertainment may be stated as either a strong or a weak restriction on the tests. A strong restriction would require that the probability of ascertainment be constant throughout the population. A weaker restriction would merely require that the probability of ascertainment of an individual not be correlated with the number of affecteds in the family. There is merit to the latter. For if the probability of ascertainment is a function of severity of disease then while the strong restriction would not hold, the weaker one might even though siblings are correlated in severity provided this correlation is genetic.

Now I should like to address myself to Dr. David's comments. I hold no brief for the terminology which I used which happens to be the terminology recommended by Bailey (Ann. Eugen., 1951). However, it does seem time that some standard was adopted. The second point is I heartily support Dr. David's remarks on the utility of single selection. It is unfortunate, however, that single selection has been often used without recognition of this fact. Lastly, I should like to reply to Dr. Lush's point as to why foster sibs are not more frequently studied. The reasons are twofold. The first is the problem of rapport which need not concern us. The second, however, involves the selection of foster parents. In Michigan, it is the policy of the adoptive agencies to place children in childless homes. This militates against those comparisons where the environment is held relatively constant such as a comparison of parent-offspring with

foster parent-foster offspring. A more serious objection arises from the fact that the placement of children in foster homes is not random. Every effort is made in placing a child to match the color of the skin and eyes, general intelligence level, nationality, etc. of the prospective foster parents. Eventually probably even blood types will be matched.

DISCUSSION

GREEN: The discussants for Dr. Spuhler's paper on "Continuous Traits" are Dr. Comstock, Dr. Dempster and Dr. Lush.

COMSTOCK: I consider it a little questionable whether a person who does just the things I do will contribute very much without considerable thought to methods in this very difficult field. My own work is in quantitative inheritance in corn and specifically to devise means for doing the sort of thing Dr. Spuhler wants to do. Corn is a very easily manipulated organism compared to humans. In two particular respects is it more flexible. One is the fact that by design of experiments we can come much closer to assuring absence of correlation between genetic and environmental effects. The other is that matings can be directed pretty much at will.

In thinking about quantitative inheritance in humans it may clarify things to first list what we can hope to learn about the inheritance of a quantitative character in any organism. It seems to me that this boils down to about three or four principal items. The first and most familiar is to learn the fraction of total variability in a population that is genetic in origin. In the past the question of whether there were any genetic effects was frequently raised. We are past that stage. The geneticist is now satisfied that all phenotypic manifestations are conditioned in some degree by genotype. Beyond the question of the relative magnitude of genetic effects lies the question of nature of gene action. In simple situations involving few genes and little effect of environment the genetic mechanisms can be analyzed in terms of the individual and joint effects of specific genes. On the other hand in quantitative inheritance the most common and generally accepted hypothesis is that many genes are involved and that effects of environment are important. Assuming this the prevailing situation, we cannot hope with known methods to provide the sort of genetic analysis that is possible in simple situations. As one alternative we attempt to throw some light on the genetic mechanism by partitioning genotypic variability into additive and non-additive portions. In these terms two others things we can hope to learn about the inheritance of a quantitative character are (1) the fractions of genotypic variance that are additive and non-additive, and (2) the

portion of non-additive genetic variance that is due to dominance. Both of these things may vary between populations. Finally, we should be able to learn something about the magnitude of effects of interaction between genotype and environment.

My impression is that in quantitative inheritance in man we have some information relative to the first of these items, the relative magnitude of total genotypic variability, but little beyond that. My further comments will be restricted to two specific suggestions concerning procedures for estimating additive genetic variance in man. I might say at this point that interest in additive genetic variance revolves around the fact that it is the portion upon which response to mass selection is dependent.

All procedures for estimation of genetic variances are based on correlations between relatives. The task is simplest when such correlations can be assumed to be entirely genetic in origin. A major problem in working with data on humans is that usually environment varies between families so that there are environmental as well as genetic contributions to the correlations. It may be that living habits and conditions are so uniform for this Indian population that environmental contributions to correlations would be insignificant but that will not generally be the case and probably should not be assumed without question in this instance.

My specific suggestions relate to estimation of heritability in terms of parent-offspring correlation. Heritability as used here refers to additive genetic variance expressed as a fraction of total phenotypic variance. Actually I will speak in terms of regression of offspring on parent father than in terms of correlation but this is a minor point. In data on random members of a population the correlation and regression coefficients estimate the same thing; the regression coefficient owes its more extensive use to the fact that the value of the correlation is affected if the data involves a non-random sample of parents whereas the value of the regression coefficient is not. If environmental effects on parent and offspring are correlated the covariance between parent and offspring, which is the numerator of the regression coefficient, will be increased. This means increase in the regression coefficient and a resulting upward bias in the estimate of heritability. When such bias is suspected satisfactory interpretation is possible only in extremes. At the one, if the regression is zero or very low one can conclude that heritability at most is very low. At the other if the regression coefficient exceeds 0.5 it is clear that it has been biased upward as a result either of environmental contribution or assortative mating. It would appear that the latter, could be corrected for through multiple regression where regression on one parent is estimated on the basis of effect of the other parent held constant.

The same problem exists in domestic animal data collected on more than one farm because environmental conditions vary between farms, and parent and

offspring are usually located on the same farm so that environmental correlations result. To circumvent this difficulty Dr. Lush in 1940 suggested that heritability be estimated from regression of offspring on dam computed on an intra-sire basis. The reasoning was that this would eliminate most of the environmental correlation because dams mated to any given sire and their offspring by that sire would for the most part be located on the same farm and in large part would be contemporary so that effect of time trends in environment would be minimized. My first suggestion is that in dealing with human data this end might be accomplished by computing parent-offspring regression within parent families. This should remove environmental contributions associated with inter-family environmental variation though some contribution associated with intra-family environmental variance might persist. It seems unlikely that the latter would be of much consequence except when the age of sibs differed greatly so that intra-family time shifts in environment could become a factor. The objection to this suggestion is that genetic variance among sibs is less than among non-sibs with the result that the regression estimate must be quadrupled instead of doubled to provide an estimate of heritability. The effect is that something like four times as much data is required to give the same precision as obtained with the usual estimate. This would be the price to be paid for eliminating bias from environmental contribution to the regression.

My other suggestion relates directly to Dr. Spuhler's case or similar ones. It has occurred to me that data involving families for which the parent grew up on the reservation but moved out and raised their own children off the reservation might yield parent-offspring regression estimates quite free from environmental contribution. The rationale of the suggestion is that the correlation in environment between parent and offspring that ordinarily results from a tendency of parents to provide an environment for their children similar to their own childhood environment would hardly survive the rather drastic shift from on- to off-reservation way of life. I don't know whether such data are available for Dr. Spuhler's population but think that if they are it could prove rather useful to use them as just suggested.

DEMPSTER: Many investigators must have worked for long periods to collect the data presented; fortunately the measurements obtained are part of a comprehensive project and are of interest in many ways; from the standpoint of obtaining estimates of heritability the data are, unfortunately, very sparse. Inspection of the charts that were thrown on the screen show that the estimated parent-offspring and offspring-offspring correlations within sexes are low and very likely insignificant; certainly at best the margins of sampling error are very wide. However the same number of observations, were the real correlations much higher, could yield estimates with much lower sampling

errors; inspection of scatter diagrams of some of the remaining forty or so measurements might therefore be worth the trouble. A very high heritability, if its exists in certain measurements, might be demonstrable with reasonable accuracy. It is conceivable that ratios or differences of two measurements, each of which might be similarly affected by environmental variations, would thereby have a higher heritability. However previous studies on other populations suggest that height itself is frequently rather highly heritable.

Differences among correlations, particularly for parts of the population, must of course be even less precise than the correlations themselves. Thus, for the characteristics illustrated, little hope can be entertained for estimating the proportions of genetic variances that are additive by comparing correlations between close and distant relatives, or by comparing sib correlations with correlations between parents and offspring. I think we must conclude that the sample, though amazingly complete and very large by many standards, is nevertheless too small for estimates of the degree and nature of the heritability of the traits discussed.

There are some additional difficulties in the present data often absent in populations of domestic animals. In many of the latter the animals are born in definite seasons permitting within-season analyses; in the human case the generations are continuously overlapping. If season is ignored, temporal trends of completely non-genetic origin could result in both parent-offspring and sib correlations. Environmental trends, or irregular variations, cannot of course be assumed absent. Thus conceivably the gradual adoption of refined foods, or a gradual exhaustion of food resources in comparison to demands made upon them might affect many physical measurements. Allowances for trends or irregularities are of course possible, although at some cost in degrees of freedom.

Means might be examined with somewhat greater optimism than variances and correlations. It is certainly not precluded on sample size alone that temporal shifts of means, or differences between groups, might not exceed considerably their standard errors. Thus any demonstrable difference between means of inbred and non-inbred individuals would surely be of interest; the degree of inbreeding however is rather slight, even though relatively large for human populations. In this connection, the higher raw correlations for inbred groups, both parent-offspring and offspring-offspring for stature as well as head height, may be suggestive. As pointed out by Dr. Comstock these correlations, for the lumped data, are mainly due to the mean differences between males and females. Apparently, then, the differences between men and women are greater among the inbred families; whether statistically significant is another matter, although the correlation differences are fairly large and consistent in the four comparisons, and a more direct examination of the sex differences might hold some promise of interest.

Were it possible to obtain heritability estimates of a number of characteristics, these would undoubtedly be of considerable interest in many respects. Aside from implications respecting the rates of change that might be expected to occur under selection, there would also be the theoretical possibility of estimating in a rough sort of way the possible relationship between skeletal remains obtained from different sources. If the observed differences between two such groups were mainly in characteristics known to have a low heritability, and thus presumably sensitive to environmental influences, the two groups would perhaps be closely related genetically; whereas if significant differences were observed in characteristics known in a number of populations, to have high heritability, there would be an implication of genetic diversity between the two groups. It would also be interesting to compare, for different populations, the total variance, and if possible the genetic and non-genetic portions separately, of various characteristics. Despite the interest of such figures, the present data indicate the difficulties of obtaining such information from relatively small populations.

It was suggested that genetic analyses of continuously variable traits are made on the basis of assumptions that cannot themselves be tested. This statement is unfortunately true, at least with respect to details of hypothetical models used. It was further suggested that the usual model was that of a large number of gene pair differences with slight individual effects, the loci concerned being widely distributed over all the chromosomes. I believe less restrictive hypotheses are permissible. Some models based on one, two, or three gene pairs, with equal or unequal effects, have very similar properties to the more extreme one mentioned, both with regard to analysis for estimating genetic variance and heritability, and for estimation of gains from moderate selection pressures. This is probably fortunate, for it seems likely that genes with large individual effects may frequently be involved in continuously variable characters. Consider for example a population whose genetic variance is due to 100 gene pair differences with equal and additive effects. A single gene pair difference, operating by itself, would then result in $100/\sqrt{10}$ or one tenth of the standard deviation of that due to all the genes working together. Probably a more realistic assumption for most populations is that of considerable unequality in the relative effectiveness of different pairs; it is not unlikely therefore that in many actual cases the gene pair with the greatest effect, if it alone were segregating, would result in a great deal more than one tenth of the genetic standard deviation actually present.

LUSH: First a detail about this Indian population being founded in 1868. Since this is 1953, that was only 85 years ago and 8 generations sounds like only 10 years to the generation, which is incredibly short. Dr. Spuhler explained that the generation interval was about 19 years, back in 1800. I take it that the

8-generation line is probably the maximum that was traced and probably starts with the first two or three generations as individuals already old, or at least mature, in 1868. In the white part of the United States' population the average generation interval is about 29 years, and has not changed much in the last 70 years. I would refer you to the Scripps Institute of Population Research at Oxford, Ohio, for details about that.

That the sex difference was in the correlations has already been mentioned. The correlations for the different sex-combinations could be pooled to summarize the evidence more concisely. The standard errors of the correlations as given will be of the order of .12 to .24 and, if all the information on offspring-offspring and on parent-offspring resemblance had been thrown together, the standard errors would still have been of the order of .06 to .12. The necessity of large numbers to locate at all definitely the real magnitude of those correlations simply cannot be avoided. When studying resemblance between relatives, you can do whatever is convenient about expressing it as correlation or regression or ratios of variance components. If you want some vivid illustrations of how much a correlation can be altered by having in one table two sexes or other groups differing in means, you might look up an old Texas Agr. Exper. Station bulletin, No. 310, published about 30 years ago.

In the introductory part of his paper, Dr. Spuhler says that the multiple factor explanation of quantitative inheritance is vague. Frankly, I don't see how it is more vague than single factor inheritance except that in the latter case you do postulate and name a factor. In either case you say that if proposition *a* is so, then *b* and *c* and perhaps *d* will follow. If you can find the data in either case, you can test your hypothesis. In a multiple factor explanation you merely refrain from naming the genes. I question whether there is such a gulf between quantitative and discontinuous inheritance as is often implied. At least when you come to decide what, if anything, you are going to do about it, I don't know that you are in any better case with single factor than with multiple factor hypotheses, particularly if much environmentally caused variance is also present.

Naturally the first gene we find when we start to study a character genetically is likely to be a gene with a big effect. If we don't experiment too long, we publish our paper and it goes into the literature as a one-gene character, but if we study it further we usually find modifiers.

The inbreeding in Dr. Spuhler's data is low. The maximum is a little less than 10 percent, which is less than results from one generation of half brother-sister mating. The effects of inbreeding would have to be extremely large and clear-cut before we could establish them on such data. Essentially the evidence is the regression of phenotypes on inbreeding. Establishing this accurately will require either large numbers or a wide range from lowest inbreeding to highest, or both.

In this quantitative inheritance, we keep coming back to the fact that in any interpretation of genetics in man or any other animal, we are comparing the resemblances between relatives or the differences between them and the differences between non-relatives. Measuring and explaining the differences between organisms related by descent is the basic subject matter of genetics now, as it was when genetics was first christened. Relatives can resemble each other because they are grown under the same environment. I suppose that discounting this is more difficult and more important in the genetics of man than in any other organism because of man's long childhood and the tendency for children to be reared in the same family. If differences in environment from family to family are important, we would expect strong resemblance between sibs because of that. Galton in his studies naturally selected certain characteristics which were easy to work with because they were not much affected by environmental variations and were in populations where the mating system was not far from random.

Discounting the environmental element in the resemblance between relatives is generally the most difficult part of estimating the heritability of a characteristic. This is likely to be even more difficult in man than in most organisms, because correlations or interactions between environment and heredity are frequent. As an illustration, consider a characteristic such as a measure of the individual's intellectual achievement, which will have been influenced by the amount of reading the individual has done. To the extent that this is determined at all by genes, children of parents who read a lot will not only receive many of the same genes which will tend to make them also read much, but in addition they will have been reared in homes where they see their parents reading and enjoying it, and where many books and magazines are available. Opportunity, precepts, the examples of their parents, and the natural inclinations caused by their own genes, will all tend to make them develop in the same direction. This environmental effect, reinforced by the correlation between heredity and environment, could easily add a large component from social inheritance to the purely genic resemblance between parent and offspring or between full sibs. Yet what would appear to be purely social inheritance, when considered from only one generation, would in turn have been partly caused by genetic differences in the preceding generation. Something of the same sort of tangle is encountered in other mammals for purely physical characteristics which are influenced strongly by the nursing ability of the dam but in most of these the difficulties are less than in studying the genetics of mental, emotional and social traits in man.

I do not understand why people interested in human genetics do not make more use of studies of foster and own children, somewhat after the manner in which Barbara Burks attacked the problem. Her methods still seem rather

productive, perhaps more so than merely accumulating more information on the correlation between relatives.

I suppose we will never get entirely rid of the widespread popular impression that if a characteristic is perfectly hereditary, the parent and offspring should be exactly alike and that full sibs should be perfectly like each other. Mendel's law of segregation should have rendered that idea obsolete long ago, but the implication continually recurs in popular discussions of heredity. With reference to the cancer studies being discussed yesterday, suppose that such cancer was perfectly heritable in the simplest manner, what difference would you expect between the percentage of cancer among the full sibs of your index cases and the percentage of cancer among the full sibs of your controls? Only about 50 percent, with some corrections for the peculiarities of percentage data. If the responsible genes have some dominance, the expected difference would be less. If there is much gene interaction, and (as is reasonably certain) some influence of environment, the expected difference might be small indeed. The point is that perfect heritability does not mean perfect likeness. In any popular publication where a word or phrase will help dispel that illusion of perfect heritability meaning perfect likeness of full sibs or of parent and offspring, I think a little such effort is desirable.

This matter of trends of human stature is one of the puzzling problems in genetics. Man is currently getting taller at a rate of about one inch per generation in countries as diverse as Sweden and the United States. Several things make it seem unlikely that much of this is caused by changes in nutrition.

The number of genes affecting a character has little effect on any procedure to be undertaken in applied plant or animal breeding or on any statistical description of the population, except whether the ultimate range of genetic possibilities is far wider or only a little wider than the present actually observed range in the population.

SPUHLER: I should like to answer one of two questions brought up in the discussion. The possibility was mentioned of comparing Indians who had left the reservation with those who stay on the reservation. This would allow us to check members of the same population in two fairly different environments. For the Ramah population the chief difficulty is that relatively few members of the group have lived off the reservation over an extended period of time. However, another useful comparison might be made between the acculturated and the non-acculturated members of the population. The classification into acculturated vs. non-acculturated members can be made fairly consistently by independent workers. The criteria include such items as whether they have gone to school, how far away from Ramah they have travelled, knowledge of English, to what extent they participate in non-Navaho activities and values.

We have some preliminary evidence to suggest that the non-acculturated Navahos have higher score on the Scheider Test of Physical Fitness than acculturated Navahos. Beyond this we have not tested for biological differences between the two subgroups. I think classification of the population into subgroups with different environmental experiences would be a more feasible approach than an attempt to locate and observe former members of the population who have left the reservation.

With reference to the length of generations, Dr. Lush is correct in assuming that 8 generations in the period 1820 to 1950 is the maximum for the Ramah population. The members of the 8th generation are still babies. But 130 years divided by 7 gives about 18 years and that is still short for human generations. The mean age of a Ramah Navaho woman in childbirth, based on about 1,000 births, is 26 years. The youngest known Ramah mother was 14 years old.

As a closing comment I would like to mention again the few major genes idea vs. the large number of genes idea as an explanation for continuous morphological variations in man. As an anthropologist, I am very pleased that more geneticists now believe it possible, and perhaps even fairly easy, to study the genetics of human normal morphological variations. I am not yet convinced that we know any cases where a continuous morphological variation can be explained by a few major genes. Dr. Glass' "hitchhiker's thumb" may be such a case. We have examined a number of possibilities in the Ramah Navaho material. For example, Dr. Gordon Allen and I classified about 200 Indians from 70 families as to whether the tendon of the peroneus tertius muscle was absent, small, medium, or large on right and left sides. If we assume that the major portion of the variation in this muscle is genetic, and if we make appropriate assumptions about reduced penetrance and variable expression, we can make the data fit a single allele hypothesis. We have not published the full report on the peroneus tertius variation because we felt the original assumption of genetic control was the very thing we were trying to demonstrate.

During lunch, Dr. Dempster and Dr. Glass pointed out that if heritability tests show a continuous variation to be under genetic control then testing for simple modes of inheritance is more tenable. But I believe there are no cases in Drosophila or the mouse where the variation of a continuous normal morphological trait has been shown to be controlled by a few major genes. This makes me a little skeptical about the possibility of demonstrating such cases in man.

STEINBERG: What I have to say is not pertinent to the last discussion. I have been accused of being academic. While I have no objection to this, I think in this case the accusation is inaccurate. I do not always work with characters that are clear-cut and uniform. I have studied two or three that are neither clear-cut nor uniformly expressed. For example, diabetics are not uniformly affected. They run the gamut from those who are completely unaware of the

presence of their disease to those that come into the clinic in diabetic coma. Nor do they all come to the clinic for the same reason. For example, we asked 2000 diabetic people why they came to the clinic. Only 40 percent came because of diabetes or symptoms associated with it. The other 60 per cent (most of whom knew they had diabetes) came for reasons other than diabetes. There were among these a number who did not know they had the disease. The reasons for coming were very varied including not a few who said, "Well, I accompanied my friend (or relative) and I travelled several hundred miles to get here so I thought I would have an examination."

Another point I meant to mention this morning concerns families coming to your attention because of inquiries made by individuals not suffering from the disease in question. I can extend the argument. I have never studied a family that was not brought to our attention by referral via a healthy individual. They were all referred by a physician other than the one associated with me in the study. I think this is quite a common way of ascertaining a family. The point I wish to make is the same one I made earlier, namely, if the probability of ascertaining families of the same size with the same number of affected individuals is essentially equal for all such families, the minute details of the method of ascertainment are not of vital importance.

TWIN DATA[1]

Moderator: Dr. Frederick Henry Osborn, The Population Council, Inc.

OSBORN: I wish to make a brief announcement before opening the session. There is going to be a world conference under the auspices of the United Nations and the United National Populations Commission in Rome from August 31 to September 9, 1954. The Population Commission of the United States, of which John Durant is the head of the secretariat, is working closely with the International Union for the Scientific Study of Population Problems. They are planning the program. The program is practically complete and will include two sections of interest to this group. One section will consider fertility and intelligence. This was set up as a separate section as the result of the interest of the British group who have been studying Scottish school children. They have contacted UNESCO for a conference this coming February on fertility, which will be a 4-day seminar preliminary to the world population conference.

The second is section 10-B, called "Relation of Population Changes to the Distribution of Genetic Factors." I might say that this world population conference is the largest thing of the sort ever planned. The quantitative problems alone of world population have become so critical in many countries that they are under very active consideration and thought by the governments. Eight years ago my friends from India felt one couldn't discuss the population problem for fear of losing one's job. Now the Indian Government has put up a large sum of money for study of a possible solution of the tremendous increase in population.

One reason for the conference being held in Rome is that the Italians were anxious to hold a conference in view of their own population problem. The Vatican has a very real interest in this problem, just as it had an interest in the Genetics Conference during which the Pope made an important address. We hope there will be value in having the conference in Italy. The interest is such that the Italian government has subscribed $25,000 to the conference. One Italian manufacturer has subscribed $25,000; the Ford Foundation has subscribed; the Rockefeller group has subscribed; and we have an indication that there will be brought together here a great many people from all over the world. The session of particular interest to us is this section on the relation of population changes to the distribution of genetic factors. This will include three sections, each section being a brief summary of five or six papers which will be prepared for each section but which will not be read because it would be impossible to handle that many presentations.

[1] Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

This is a session of considerable scope and many of us will get letters from the organizers of the session asking advice particularly on overseas personnel—personnel from other countries. We want as much as possible to make this session feature men from other countries.

The subject of the afternoon discussion is "Twin Data", and will begin with a paper by Dr. Kallmann.

Twin Data in the Analysis of Mechanisms of Inheritance[1]

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HAVING returned only last week from a tour de force through European twin research centers and conventions, I have had little time to prepare a review of the methodological aspects of twin studies. I would like to think, however, that the fruitful observations made in Western Europe during the past two months will compensate for certain shortcomings in my report.

From my general impressions of current European research activities in the field of twin studies, I would say that the overall balance sheet looks rather favorable. On our side of the Iron Curtain, at least, professional interest in the problems of human genetics is growing everywhere, particularly in medical specialty groups. Increasing recognition of the potentialities of statistically representative twin data has certainly played a considerable part in the resurgence of a science which, after yielding to heavy political pressure on several fronts, was eclipsed for a time.

The most striking evidence for the strong revival of interest in medical genetics in the free European countries was the international symposium held at the opening of the new Gregor Mendel Institute for twin research at the University of Rome. During the impressive inauguration ceremonies, a policy-setting address was delivered by Pope Pius XII, in which the need for systematic and ideologically unshackled research in human genetics was stressed.

One of the highlights of this forty-five minute address, delivered in French[2], was the following passage concerning research in human genetics and its important practical applications: "Neither on the part of reason nor on the part of thought inspired by Christian teaching are any barriers placed in the way of

[1] Presented at the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

[2] The English translation quoted was kindly supplied by the Vatican Press Office.

research for truth or of its attainment or its affirmation. . . . Experience shows that natural dispositions, whether good or defective, exert a very strong influence on the education of man and on his future conduct. Undoubtedly the body with its aptitudes and its organs is only the instrument, while the soul is the artist that plays on the instrument; undoubtedly the ability of the artist can compensate for many defects of the instrument; but one plays better and more easily on an instrument that is perfect; and when its quality descends below a certain limit, it becomes absolutely impossible to use it. . . ." I am personally convinced that the ideas expressed at this congress will have a far-reaching effect.

As for the work which will come out of this magnificent new Roman institute under the direction of Dr. Luigi Gedda (Mayer, 1953) its staff is in the enviable position of being able to make a substantial contribution to the progress of human genetics. Indeed, if the quality of the research conducted there can match the architectural splendor of its walls and the ingenuity of its planners, it will soon prove a challenge to the rest of us. With our less spectacular research facilities, we will have to redouble our efforts to hold our own in what was described in the papal address as "perhaps the most dynamic research" of our time.

Unfortunately, the very twin studies which have revitalized interest in human genetics have brought to the field a great number of controversial issues and methodological obscurities which exist in disciplines dealing with man. In the final analysis, there is no difference between a twin and a single-born individual, once they have come through the ordeal of being born. In other words, any selective process, any specialized technical jargon or any investigative procedure, used in the study of the individual, can also be applied to twins. Consequently, a discussion of the complexity of twin data could conceivably take in the entire range of problems peculiar to disciplines concerned with the biology, psychology, chemistry, and social aspects of human existence.

For the purposes of this report, therefore, it is necessary to distinguish experimental studies based on the application of the various versions of the twin-study method from two other sets of data: (1) incidental observations in persons who happen to be twins; and (2) technical investigations of the twinning phenomenon itself. For obvious reasons, this report will be confined to the applications of the twin-study method.

In view of the multitude and complexity of problems encountered in studies of the total variability in man's adjustive potentialities, it is fortunate, indeed, that the majority of these variations can be investigated by means of the twin-study method. Naturally, this method cannot be expected to solve all problems. But the fact that it has certain limitations (which will be discussed later) should not prevent its widest use in those many fields where it is applicable.

As far as the classification of one-egg and same-sexed two-egg twin pairs is concerned, the modern similarity method has replaced the fetal-membrane method. There is no longer any question that one-egg pairs are not all born with one placenta (Gedda, 1951; Lamy, 1952). Apart from such usually variable physical characteristics as facial features, dental specifications, hair color and texture, color of the eyes, body size, and so forth, dermatoglyphics and blood factors are now regarded as the most reliable criteria for distinguishing the two types of twins. Fingerprints can be analyzed qualitatively as well as quantitatively. Even the qualitative analysis is usually sufficient for the practical purposes of the similarity method, and by means of quantitative procedures, dermatoglyphics alone guarantee a maximum degree of reliability. A detailed discussion of the procedures used may be found in the publications of Cummins and Midlo (1943), Slater (1953) and Von Verschuer (1949). Ford Walker, too, has accumulated a wealth of dermatoglyphic data which, when published, will be very helpful.

Regarding the use of hematological tests, it may be mentioned that twins cannot be monozygotic if the blood groups are different. If the blood groups are the same, they may or may not be monozygotic. Assuming that a given pair is dizygotic, a possible way of proving it hematologically has been provided by Wiener (1952). By this method, the chances of proving dizygocity by the three M-N types is 40.5 per cent, by the three S-s types 36.7 per cent; for the nine M-N-S types the chances are 56.2 per cent. The conditions under which these figures are applicable, have been specified.

According to Race and Sanger (1950), the chances of two twin partners being monozygotic or dizygotic can be estimated with the aid of a statistical procedure which assesses the probability of identity of same-sexed twins with the same blood groups. The chances for every twin family are calculated separately, so that the amount of hematologically verifiable information is apt to vary considerably from family to family.

Obviously, this part of the diagnostic work is important in a certain number of twin pairs, and should be done only in laboratories specializing in blood-group testing. It is equally clear, however, that in many instances the diagnostic and clinical emphasis must lie in other directions. Hematological tests are disproportionately expensive, depend on the availability of both twins, and can often be dispensed with, especially with respect to important traits which, by their very nature, take the research subject out of the reach of laboratories.

Generally speaking, the twin-study method can be used for the investigation of many genetically significant and clinically definable traits, provided that they lend themselves to adequate sampling and zygocity classification. In fact, the practical application of the method is so flexible that it must be decided in every instance which of the three main versions promises the best results.

In some cases it is possible to limit observational or experimental twin data

to a few selected one-egg pairs whose aptitudes, physiological reactions or emotional adjustments are compared under different life conditions or in response to different methods of therapeutic management. This method used especially by Gesell and Thompson (1941) is called the "cotwin-control method."

The second version consists of the original twin-study method which requires the collection of a representative series of one-egg and two-egg twin pairs of either or different sex with respect to a trait to which the investigative principles of the proband method can be applied. In this procedure, the comparison of observable similarities or dissimilarities is limited to twin subjects.

The third version of twin studies involves various combinations with the statistical principles of the census, proband and sibling methods or with special pedigree investigations. In this manner, twin data may be used as an economical substitute for a total population survey, or to combine the advantages of thorough family studies with the application of test procedures which require experimentally controlled conditions. The former method is indicated when there is a need for investigating intensively and on a long-term basis a numerically limited but otherwise representative sample of twin subjects, rather than an entire population investigated less thoroughly and only for a brief period. The other method is particularly useful when a simultaneous and well-integrated analysis of a certain trait is called for by the apparent interdependence of multiple factors of causation. Both modifications of the original twin-study method have been referred to as twin-family method.

The main advantage of the last procedure is that it extends the number of genotypically dissimilar sibship groups which can be compared under similar conditions of culture and home milieu. The six distinct categories compared in this manner are: one-egg twins, two-egg twins of the same sex, two-egg twins of opposite sex, full sibs, half-sibs, and step-sibs. Where a representative sample of twin index pairs with complete sibships can be collected, this method provides the broadest possible scope for analyzing the relative effects of genetic and non-genetic factors in relation to a great number of physical and mental traits.

The advantages of the twin-study method may then be summarized as follows: (1) It constitutes an excellent sampling procedure for the study of variations displayed by different genotypes in a controlled environment or by a constant genotype under the influence of different environmental conditions. (2) In the investigation of traits requiring close personal contact with research subjects from various segments of the population, the twin-study method provides an innocuous approach to families who might not otherwise be willing to give information about their private lives. (3) For traits which need long-term observation under controlled conditions, the method is more effective, flexible, and economical than any other procedure dealing directly with human beings.

The main limitations of the twin-study method are due to a number of unavoidable circumstances. Obviously, twins cannot be separated before they are born, nor can they be provided with two mothers of different age, personality, or health status. Also, two-egg twins are no more dissimilar genotypically than brothers and sisters and, like them, are rarely raised in different cultures. Therefore, even fraternal twins are unlikely to fall into the extremes of theoretically possible genetic and cultural differences.

Furthermore, with respect to most human traits, it is not possible at our present state of knowledge and technical equipment to assign exact quantitative values to the relative contributions of genetic and environmental factors in the production of individual differences. The average difference between one-egg twin partners is no precise measure of environmentally produced variations, nor does an increase over the average difference between two-egg twins represent the exact contribution of genetic influences even in relatively comparable environments (Kallmann, 1953).

In fact, only under rare and exceedingly rigorous experimental conditions can the environment of a human being ever be separable from the hereditary components of his existence. In many instances the individual selects, and therefore determines in part, some important aspects of his environment. The long-postulated type and degree of causality in the relationship between heredity and environment may often be reversed.

Another limiting factor has recently been stressed by Darlington (1953). In the long-term heredity of the species, the cytoplasm of the fertilized cell may be of relatively little importance, but it may not be without significance for the phenotype of the individual. In one-egg twins, for instance, a certain degree of discordance may be produced not only by differences in the pre-natal environment, but also by the action of genes which may be sensitive to cytoplasmic asymmetry. Thus one-egg twins, while they may pass on the same heredity to their offspring, have not necessarily received the same heredity from their parents. Discordance between them is not, as is commonly assumed, a measure merely of post-natal or even of pre-natal environmental effects; it also may have a genetic component.

One further difficulty with twin studies is that, more than any other kind of experimental work, they cut across the customary borders of individual disciplines and require the prolonged and expensive maintenance of specialized research teams trained to deal with human subjects. Ideally speaking, individual workers or teams should properly delineate their tasks according to whether the variations studied fall into the normal or the pathological ranges of variability. Unfortunately, however, the dividing lines are seldom clearly defined, and one does not easily find research workers who are willing to anticipate limitations in their investigative capacities.

As a result, some twin researchers have recently been misled into describing

observed temporary and perhaps easily reversible dissimilarities between the behavioral patterns of twin partners in the antithetic setting of absolute dichotomies, or in mystifying terms borrowed from transcendental schools of thought. Such erroneous evaluation of twin data is most likely to arise where it is necessary to distinguish between causes and motivations of human behavior.

Finally, may I reemphasize that the twin-study method provides us with an investigative procedure which can be applied to many important problems of human and medical genetics. However, the proper application of the method requires wise selection of topics, and the concentration of available facilities and personnel in a few strategically situated research centers. Instead of encouraging needless duplications of studies limited in scope and procurable support, it would be well to devote all regional resources to the organization of a few broad studies, and the training of men qualified to handle them. Our objective should be not only to demonstrate that hereditary factors play an important role in many normal and abnormal behavior variations, but precisely how this action takes place.

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DISCUSSION

DR. ALLEN: As Dr. Kallmann has described the twin family method, it combines the older style of twin study with the usual family method, and it has the great advantage of obtaining both types of data for one set of probands. How-

ever, in that form it adds nothing new, and I should therefore like to draw attention to a modified analysis of twin family data which may sometimes contribute unique information. Although this method has been used in a few instances, it has apparently not been recognized as a method in its own right and it has certainly not been applied in all studies where it was appropriate and where the necessary data were available.

It is likely that for many medical conditions the same clinical picture may be produced either by heredity or by environment. In such a case, a sample of single-born probands will contain a proportion of the environmental cases, or phenocopies, and a family study has to start out by lumping the relatives of both types of case. If, however, one starts with monozygotic twins as probands, a separation of the concordant and discordant pairs will in general segregate the genetic cases from the phenocopies. This permits from the outset a separate analysis of two groups of relatives practically without reference to familial incidence, so that the latter can be studied as an independent variable in the cases presumed to be genetic. Even with a very incomplete separation of genetic from non-genetic cases, the method may bring to light important statistical differences between the two groups of relatives. At the very least, this method of analysis provides another type of internal control in twin studies.

A second point I wish to make is in regard to zygosity determination. Without doubt blood grouping is very valuable for this purpose and, as a tool, it warrants exhaustive study at the present time. But I do not think that it ought to be accepted as an absolute requirement for every twin study. First, as Dr. Kallmann said, it is relatively expensive. For a complicated study on a small number of twins the expense may be negligible, but when you have to pay a laboratory to do blood grouping on a hundred or more pairs of like-sex twins you acquire more respect for this as a budget item. Second, blood grouping provides little positive evidence for monozygosity unless you can also type a number of family members and unless, further, these relatives have a fortunate combination of blood types. Third, some workers share with me a growing suspicion that, even when you find such a family, the possibility that the twins are dizygotic is greater than current probability calculations indicate. Perhaps this suspicion will not be confirmed, or perhaps blood group chimeras are more common among human dizygotic twins than the two instances now known would suggest. In any event, it seems that caution is advisable, for the present, in using blood groups as positive evidence for monozygosity. For these pairs and even for many dizygotic pairs certain other criteria are more useful and must be emphasized. This applies particularly to dermatoglyphics, which has a potential usefulness in twin studies that is, I think, still largely unexplored.

Finally, I should like to comment on terminology. We have heard here at least three different adjectives to describe twins derived from two zygotes: dizygotic, dizygote, and dizygous. The last, because of its closeness to the

words, "homozygous" and "hererozygous", has been responsible for several misunderstandings among non-geneticists, within my own experience. Perhaps some decision could be made about these terms the next time human geneticists gather to discuss nomenclature.

DR. NORMA FORD WALKER, M.D. (*University of Toronto*): Speaking of the twin method generally, my criticism is that too much confidence is frequently placed in twin studies where the diagnosis of zygosity is inadequate. I doubt that we have yet reached the point where the diagnosis of zygosity can be made both easily and with certainty. It is true that some monozygotic twins are so alike that they can be recognized with little error and that some dizygotic twins are very different. But between those two extremes there is a large overlap. Merely comparing finger prints will not distinguish the two types of twins with certainty.

Uniovular twins form a graded series, beginning with those who are much alike, grading into those who are less alike, and ending with the conjoined twins who are frequently so different that it was once thought that they were formed by the union of two separate embryos.

For a sound diagnosis of zygosity a battery of tests should be used and these tests should be reported in the published account, so that others may evaluate them. The dermatoglyphics used should include not only digital patterns, but also palm and sole patterns. At Toronto we are also using complete blood groups. We are fortunate in having a grant for this purpose as well as a new Canadian laboratory in Winnipeg, directed by Dr. Bruce Chown, where complete blood groupings are done. The individual investigator will add to his battery other physical characters or chemical tests.

For adequate twin studies we need to know a great deal more about the fetal development of twins and their relation to the birth membranes. In one of our studies at Toronto we have now collected over 800 placentae of twins and injected them with liquid latex. Thus gradually we are coming to know which monozygotic twins had two separate placentae, which a single dichorionic placenta, a monochorionic with separate circulations of the blood, a monochorionic with a common circulation, or a monochorionic and monoamniotic placenta. It is proving enlightening to relate the dissimilarity of nonozygotic twins to their twinning time, the latter being indicated by the type of placenta.

If in twin studies the diagnosis is not sound, e.g. if one merely guesses whether the twins are monozygotic or dizygotic, then the whole foundation of the study is insecure and the descriptions and calculations which follow lose their value.

My plea is that all of us who are engaged in twin studies should be most critical of our work. And first of all comes a sound diagnosis.

GLASS: I want to take this opportunity to discuss the desirability of extending into the field of human genetics the utilization of twins as material, the type of experimental approach so successfully begun a few years ago by Prof. Bonnier in Sweden, utilizing twin cattle. This type of experimental approach has been copied and extended to cattle in New Zealand, Scotland, and now in the United States at Beltsville, with regard to nutrition. The method is a simple one. First, of course, you are faced with problems of diagnosis of monozygotic and dizygotic twin pairs. When you have presumably determined the monozygotic twin pairs you use one of each pair as an experimental animal and the other as a control animal in an experiment where one is kept on a standard diet and the other is fed some particular diet, and the gain in body weight is recorded.

I was asked some months ago to participate this winter in a symposium to be held by the Association for Research in Nervous and Mental Disease, because they wanted somebody to talk about the genetics of adaptability. If we stop and think, we will all agree that nothing much is known about the genetics of adaptability in the human species; yet I think we are all convinced that the human species differs from other animal species principally by reason of its very high measure of adaptability; therefore, human geneticists should be particularly concerned with the problem of genetic adaptability. In a very simple way, to start with, we can use monozygotic twin material for various types of studies—the number and type of which could be multiplied almost indefinitely —that will be very revealing in this direction.

I want to describe briefly one such study we have started at Johns Hopkins University, in order to indicate more specifically just what I mean. We had a pair of monozygotic twin boys who were seniors at the University and majoring in the Biology Department, who were very much interested in human heredity and particularly in twin studies, so we worked out various kinds of experimental approaches. Now, the type of twin methods we have heard discussed this afternoon has centered around problems of diagnosis and what I would call a comparative description of twins. They do not get into the actual use of twin material for an experimental analysis of differences that may exist in the *capacity* to respond, and the like. These twins are quite well known—they have been studied by geneticists a number of times—there is no doubt about the diagnosis, and I assume they are genuinely monozygotic. We gave them intelligence tests. We found that on their IQ's both were identical—each one had an IQ of 131. These tests were given by a competently trained psychologist. The tests were repeated, using an alternative form, after an interval of about a week. The tests were given the twins separately each time. Each twin was told confidentially just before starting in on the second test, "We don't want this to disturb you, but your brother did much better on that last test than you did." Then after a few minutes of allowing this to soak in, they were given the second,

alternative forms of the test. One twin made exactly the same score as the first time; the other made 10 points lower. Very obviously that second twin was extremely disturbed by being told that his brother had made a higher score, and believed it; while the first one told us afterwards that he just did not believe what he was told. That gets at the type of experimental approach that I suggest as offering more possibilities.

We undertook to make a little study of the responses of the twins to caffeine. The twins were not used to drinking coffee at all. As we eventually worked out the plan, it was first through development of a type of test involving muscular-visual coordination and precision. A target sheet was made on which ten small targets of one centimeter in diameter are drawn, each with a bull's eye half a centimeter in diameter. The subject is given a laboratory dissecting needle and at elbow length tries to stick the needle into the target 10 times. We scored it one point for each hit on or within the outer circle of the target and two points for each score on the bull's eye. A total possible score of 200 points can be made, and the whole test can be run through in 3 minutes, and be repeated at suitable intervals.

After some practice, the twins showed a stabilized type of behavior in which they would score about 135 on the first trial on any given day and then show a fairly steady increase over a period of about 2½ hours, and both of them followed this same characteristic kind of practice effect. The following day the performance would drop back to the original level again.

Capsules were prepared containing either 5 grains of caffeine or lactose, which looked identical. The twins were each given a capsule, one a capsule of one kind and the other one of the other kind, without knowing what they were getting. They were given tests in separate rooms where they could not observe the effects on one another. We have assembled a large body of results from this type of testing and with very consistent results. The twin that gets lactose shows the regular kind of practice effect. The other twin, a few minutes after taking the caffeine, shows a decline in accuracy of considerable extent—the score drops down to about 110 after a period of about 30 minutes from taking the caffeine. This is followed by a marked increase until at 1 hour the score made by the caffeine-treated twin far exceeds that of the other. Then there is a decline, until after about 2 to 2½ hours after the drug is taken the two twins are back on the same level again.

It would be very interesting to know whether if you did 10 other pairs of monozygotic twins they would all respond the same way. It would be interesting to know whether a random group of students of the same sex and age as these boys would respond in the same or in different ways. The experiment I have reported isn't a very complete study at the present time, but I give it as an illustration of a method to be employed for making use of twin material

that can give us a great deal of insight into the relation of genotypic constitution and environmental background to various kinds of physiological responses.

DR. HERNDON: Dr. Kallmann's summary of the applications of twin studies to problems in human genetics has been most enlightening. It would seem not inappropriate to re-emphasize an aspect of technique which Kallmann has previously discussed more extensively in his presidential address to the American Society of Human Genetics (Kallmann, 1952), namely, the value of the team approach. As a science, human genetics bridges the gap between laboratory genetics and clinical medicine, and impinges and overlaps certain areas of several other disciplines. The twin-family technique is particularly applicable to problems in which simple or complex interactions of hereditary and environmental factors are suspected. By their very nature such problems can usually be handled most adequately by coordinated attack from several aspects by teams composed of specialists in different fields. For example, work such as Kallmann has done in schizophrenia would require application of specialized knowledge from the fields of clinical psychiatry, internal medicine, clinical psychology, anthropology, statistics, biochemistry and genetics, and in addition field workers trained in collection of data of specialized types. No one person can be competent in all fields required for most studies of this type. It would be a serious error of experimental design to fail to provide for obtaining the top quality of special knowledge in any required field.

As a corollary, it may be pointed out that the twin-family method applied by a team of specialists in diverse fields offers excellent opportunities for studying physiologic genetics. It is no longer sufficient to merely characterize the mechanism of transmission of an hereditary defect, although this is an essential first stage of any investigation. In addition information is desired concerning the series of events that must lie between the establishment of genotype at conception and the full development of the inherited characteristic. In a disease where concordance is shown by a majority of monozygotic twin pairs and by a minority of dizygotic pairs, the discordant monozygotic pairs offer an unparalleled opportunity for identification of environmental conditions which may suppress the clinical appearance of disease in a person genetically susceptible. The possibilities for study of biochemical, physiologic, immunologic and other factors in such situations have been barely touched in the past, and offer opportunities which should prove very fruitful avenues for future research.

STERN: I should like to underline the desirability of making more use of foster children in order to disentangle genetic and environmental components in studies of familial incidences of diseases. While it is true that selective placement and the rarity of the material levies severe limitations on the use of foster

children the eventual gain should be worth a prolonged and, perhaps, joint effort of various investigators. The method of using spouses as controls for affected individuals is useful for the period after marriage but, obviously leaves untouched the first two or three decades of life. It may be possible to ask hospital patients routinely whether they grew up as adopted or foster children, and thus to accumulate a pool of individuals which can be studied from many different angles.

Another method involving comparisons of half-sibs, which has been used successfully by Dr. Kallmann, also deserves wider attention. The high incidence of legal "serial polygamy" provides a large source of relevant individuals.

A different topic on which I wish to comment concerns the attempt to use the term "genetic differences" in a context where it is liable to lead to misunderstanding. It has been suggested that phenotypic differences between identical twin partners may result from the possibility that the two parts of an egg which give rise to the twins were cytoplasmically different. It will be difficult to prove or disprove this hypothesis. Even if some day it should be possible to prove it correct in some cases there seems to exist no good reason why such differences should be called genetic.

Finally, in connection with the discussion of human blood mosaics, I should like to call attention to the possibility that such mosaics may not always originate in connection with twinning. Various ways are known by which genetically mosaic individuals can arise: presence of two egg nuclei participating in development after fertilization with one or two spermatozoa, participating in development of a nucleus derived from a supernumerary spermatozoon, etc. While such cases in a mammal may be rarer than in insects or birds their possible existence must be kept in mind. Often such genetically mosaic individuals would exhibit an external mosaic appearance. Occasionally, however, the externally noticeable traits may be dependent on tissues all of which were derived from a genetically homogeneous region of the embryonic germinal disc while part of the blood forming tissue may have come from cells of a mosaic embryo which were of different genotype.

SPUHLER: It is not that serological characters are important for zygosity diagnosis because they are serological but because they are the only common human characters where we can associate a known genotype with an observed phenotype with a high degree of reliability. The serology genes are valuable here because they have high penetrance and constant expression in all known environments which allow twins to survive. There should be at least one study in which exhaustive serological tests are made to diagnose zygosity. Then it would be possible to test the reliability of other methods, for example dermatoglyphics, now used to sort twins. We know that all the variability of dermatoglyphics is not due to genetic differences. The cost of making a serological

diagnosis at a given probability level can be reduced by testing parents of the twins. Until we establish objective tests for twin diagnosis, we may be involved in a circular type of reasoning: twins are identical because they look alike, and they look alike because they are identical.

DR. NEEL: In support of what Dr. Spuhler has just said, I should like to emphasize that blood group studies of twins, their siblings, and their parents provide the most objective method available at the present time for the diagnosis of zygosity. It seems to me important that at the earliest possible moment someone conduct a study wherein two groups independently rank twins, one group on the basis of the blood groups, the other on the basis of morphological findings. A comparison of the results could not help but be enlightening, no matter what the findings.

STEINBERG: Recently, Dr. Race published results concerning the blood of a woman which was found to be mosaic at three loci. A proportion of the cells was agglutinated by one set of antigens and the remainder by a second set of antigens. These proportions were the same for each of the loci. At the time of the analysis, there was no knowledge of whether or not this woman was a twin, but it was assumed, because of the data, that she probably was. Subsequent investigations confirmed the assumption. Unfortunately the twin had died shortly after birth and could not be investigated.

DR. B. PRICE (*U. S. Children's Bureau*): I agree with Dr. Kallmann on the need for caution in interpreting monozygotic twin differences in terms of posnatal influences. We should try to make the need for caution better known to all kinds of specialists studying monozygotic pairs.

I doubt whether the intra-pair differences are due as often to "mosaic" effects and cytoplasmic factors as to various other prenatal and natal conditions. But the more evidence we can obtain on any and all such factors the better. It will be important both for developmental genetics and for improving our interpretation of the data in the twin studies already published. The value of the data in those studies will not be reduced if we find some re-interpretation is necessary.

GOWEN: I am much interested in the efforts of human geneticists toward correct diagnosis of twins as monozygotic or dizygotic particularly as animal genetics is now utilizing twins in researches where accurate diagnosis is of primary importance. In 1922 I wrote a paper entitled "Identical twins in cattle?" I still have that question mark. My plea is that the impressive work reviewed today be extended and expanded. Much more embryological evidence on twins per se is required. We have the question of how monozygotic twins arise. Are

they due to the division and separation of the egg at the two cell stage? Are they the result of the budding off and separation of the twins in later stages of development? Guesses are rather far-fetched,—information on the armadillo is practically our only source. Other methods of twin formations are possible. The egg may divide and the two halves be fertilized by separate sperm. Rothenbuhler's work with bees has shown that sex mosaics are formed by the egg nucleus being fertilized by a sperm and then developing into the female parts of the organism, and a second sperm entering the egg cytoplasm developing into the rest of the organism, each genotype resulting in characteristic adult phenotypes. Should the two parts separate in development this method could likewise lead to twins. But enough has been said to indicate the importance of knowing how monozygotic and dizygotic twins originate.

This is particularly true when the monozygotic twin concept is extended to cattle, mice, etc. I would accept now and did accept earlier the conclusion that monozygotic twins are found in man, but accepted it not on anything discussed today, but on sex ratio. The human twin sex ratio approaches 1.07 male male:1.20 male female:1.00 female female. In cattle, for data representing a combination of 12 sources, the sex ratio is 0.88 male male:1.7 male female:1.00 female female. Considering that like sexed twins should equal the unlike sexed twins there is an excess of 4.3% like sexed twins that could possibly be identical. This figure is biologically small even though it is significant at the 4 in 1000 level. The excess comes in female-female twins.

Selection of identical twins in cattle, if they exist, is beset by more pitfalls than selection made in humans. Uterine environmental factors further complicate the selection. Some 90% of cattle twins show common fetal blood circulations. Antigenic proteins, probably including islands of self perpetuating cells, are exchanged by the twins. In consequence the twins are subjected not only to a common environment but to the same formative substances which guide the pattern of embryological development from egg to adult. After birth any islands of implanted cells would likewise tend to make the twins similar. Development under these conditions should make the animals alike. The similarities may extend even to characters for which the particular animal does not possess the proper genes, as for example in Drosophila, the transplantation of a homozygous vermillion eye disc into a wild type fly results in a wild type eye color, not that of its vermillion genotype. Diagnosis of monozygotic twins on the basis of the limited criteria in use and because of the factors discussed above is certainly subject to many errors. Research workers utilizing twins for nutritional, genetical, and other investigations in agriculture and physiology have accepted twin pairs as monozygotic or dizygotic rather readily considering the implications involved are of such moment that exact diagnosis is required. I am happy to hear that the human geneticists are pulling away from careless diagnosis to a careful examination of the criteria by which the type of multiple births are

separated. I urge that embryological observations on the manner in which these twins occur is of primary significance.

LUSH: I would like to speak against this counsel of despair and undue caution. Probably I am the only man here who has seen the work with identical twins in cattle at all three of the leading centers for this work: Sweden, Edinburgh, and New Zealand. The cattle workers there have already found these twins and gone ahead with the work without knowing the things you say are necessary. It is done primarily by considering all the evidence on each pair. The twins in any pair are alike in some characters and unlike in others. When one adds for each pair their total likeness and unlikeness he gets enough discontinuity in the distribution for likeness that he is rarely in doubt about whether they are identical provided many characters were observed. The cattle are observed at an early age, usually before they are a month old. This could bias or wholly prevent the study of a characteristic such as color or bone dimension which was itself used in making the diagnosis of zygosity. If one were later to study such a characteristic on these twins, he might be doubling back on his tracks. But the method does not bias the study of characteristics not yet apparent when the diagnosis is made. On human twins you can't do your diagnosis that early and wait 20 or 30 years to follow it up.

In not a single characteristic is the diagnosis based on a definitely known gene that is 100 percent penetrant. The diagnosis is based on similarity, merely piling character on character until you get enough discontinuity to make you certain. In cattle a few cases remain doubtful. Dr. Bonner estimates that they throw away about one half of the truly identical twins in these puzzling cases, merely to be sure that no truly dizygotic twins are included.

I wish also to comment that those calves are rich material on which a psychologist might work. The twins tried to stay together conspicuously when turned in the pastures. When through grazing they generally lie down close to each other. They seem unhappy if they are not together. For observing many of these behavioral characteristics it is advantageous to have each pair stabled in the barn together, instead of having their locations randomized. Then it is easy to see differences in nervousness, in the way they hold their heads, or switch their tails or lick with their tongues, etc. Of course, they may learn from each other but, if proximity were a major factor, they might as well learn from the non-twin heifer on the other side. Although I have considerable faith in randomizing as a principle, I think in this case the disadvantages outweigh the advantages.

Nose prints of cattle have been used for diagnosis and are a helpful criterion but by no means perfect. From the purely statistical point of view one pair of identical twins is worth four pairs of fraternal twins for estimating the additively genetic variance, but identical twins are uniquely valuable for estimating

the dominance and epistatic portions and the interactions between heredity and environment. They also offer opportunity to study penetrance of characteristics not used in the diagnosis of zygosity. For example, I saw a pair of identical twin bulls in Scotland, of which one was cryptorchid but the other was normal. Various earlier known facts about cryptorchidism indicate that its penetrance is not perfect.

Another thing illustrated well by this twin work is the advantage of not having all research on a given topic organized into one large project. The man who heads an Institute usually has a definite viewpoint which will often cause other theories or viewpoints or possible experimental approaches not to get full consideration. If several institutes are working independently in the same field, that is less likely. I know there can be a waste of resources in duplication but I also know that an even larger waste can be caused by not having enough replication. Most bits of research on important topics should be at least duplicated or triplicated at institutions independent enough to correct each other's mistakes with considerable zest.

My final comment from the twin work is that the evidence shows heritability to be much higher than is indicated by the evidence from parent-offspring or sib resemblance. The reasons for this are not clear. Common prenatal environment may be a factor but fraternal twins show that in many cases it is unimportant. Epistasis and dominance may be part of the explanation but certainly are not all of it for some characters. Interaction between heredity and environment may be more important than most of us have thought. "One man's meat is another man's poison" may be a more usual situation than anyone supposes. At any rate the high heritabilities generally found in studies of identical twins are a challenging problem in genetics.

My plea here is that we do not set up impossible perfect standards for the diagnosis of zygosity, but use the workable means already available, throw out the doubtful cases, with due regard for biases that might be introduced, and go ahead.

DR. NEEL: Dr. Lush, you have said that at experiment stations interested in cattle twins about half of all like-sexed twins are not utilized for investigative purposes because their zygosity is uncertain. You have further stated that hereditability estimates based on cattle twins are proving significantly higher than estimates based on standard approaches. I wonder if establishing zygosity by a method based on physical resemblances in which half of the material is discarded as indeterminate doesn't introduce a sufficient bias into such studies to account for the higher hereditability estimates.

LUSH: The high hereditability estimates that interest me especially pertain to rates of growth and to milk and fat production. You might have a slight case

concerning rate of gain as it is possible that some slight indicators of that would show at one month of age, but it is unlikely that milk or fat production would be foreshadowed in any calfhood character. Main reliance in diagnosing zygosity is on bone dimensions and shape and coat color. I don't think the cattlemen are really biased by the method of diagnosing zygosity in the things they actually are studying. If they did try to study the hereditability of peculiarities of bone shape the method would bias that heavily.

REED: I wonder if Dr. Lush would say what happens with regard to the twins associating together on pasture when they had been stabled in randomized locations in the barn.

LUSH: In New Zealand they stayed together on pasture conspicuously. The climate is such that they spend little time indoors and hence the twins spend little time side by side.

SPUHLER: A number of papers have been published on asymmetry in man. Using these results, we could get, if you like, 50 or 100 measurements or indices to show that the left side of an individual is different from the right side. By analogy with your argument, are we to conclude that the genotypes of the left and right side are different? Also, to return to Dr. Allen's case, there are instances where one eye is brown and the other is blue in the same individual.

LUSH: Isn't that a common result with spotting patterns? It happens often with "Dutch" rabbits. Spotting pattern varies too much to be diagnostic of zygosity. About 60 percent of the variance in spotting in a stock of guinea pigs was found by Wright to be non-genetic. In mice, cattle, rabbits, etc., the similarity of spotting on the right and left sides is high, the correlation in most cases being of the order of .8 to .9 or higher.

SPUHLER: Couldn't you select criteria in such a way that the two sides would be different?

LUSH: It seems to me you are implying that if phenotypes differ, the animals must have different genes. We must remember that phenotype and genotype correspond perfectly only when environmental variations have no effect; i.e. when penetrance is perfect. Often that will not be the case.

SPUHLER: You are suggesting an analogous means of classifying twins.

COMSTOCK: There is another point related to use of identical twins as subjects in non-genetic experimentation that is worth noting. The suggestion inherent

in what Dr. Glass has said is that the precision of such experiments may be much greater when comparisons are made on identical twins. Dr. Lush pointed out that the apparent high genetic variance among identical twins may be due either to much non-additive genetic variance arising from dominance or other non-additivity in gene action or to considerable variance from genotype-environment interaction. The point I want to make is that if identical twins are used as Dr. Glass has suggested the resulting data should help to resolve the issue pointed up by Dr. Lush.

Studies in domestic animals of variability among monozygotic twin pairs relative to that between members of the same pair have for the most part been based on data collected on animals exposed to what we'd commonly call uniform environment. The data is then what a statistician would call uniformity data. Variance among pairs as estimated from such data contains all inter-pair genetic variance but also the variance of whatever effects may be present as the result of interaction between genotype and environment. Estimates of this type have been interpreted to indicate that, as subject material for non-genetic experiments a pair of identical twins should be worth as much as 20 other animals. This interpretation assumes interaction variance is absent or negligible. On the other hand in actual experiments the interaction variance contributes to experimental error variance and if the interaction variance is considerable, experimental efficiency would not be increased as much by use of identical twins as is otherwise to be expected. The obvious corollary is that such experiments would provide information on the nature of the high inter-pair variance that has been noted in uniformity data. Because this is an important issue at present I feel there is additional reason to press Dr. Glass's recommendation on experimental use of identical twins. It is one way to learn something about the magnitude of genotype environment interactions.

OSBORN: If there is no further discussion, we will close the session on twins. If an individual or foundation with large sums of money were looking for advice on how it should be spent to learn the most possible about human variations, I believe we would recommend that it should be spent on human twin studies.

LOOKING TO THE FUTURE[1]

Moderator: Dr. C. P. Oliver, University of Texas

OLIVER: I want to say just a few words to open the meeting tonight. This is to be an open meeting. Any question that you have can be brought up for discussion. However, I do want to mention just a few points in the hope that we can have certain of them discussed. We are faced with these conditions in human genetics. We have to determine what we need, how we can get what we need, and then what we will do with the information after we get it. To a certain extent we have been discussing these points during the two days of meetings we have had.

We need to know more about the traits already being studied; particularly about the variability of traits, the variability within a family group having more than one individual with a trait, and the comparative condition or variability when the same trait occurs in different family groups. We have heard discussion as to the significance of variability in human genetics. Too little is known about the cause of variability. There is need for more study about interaction between genes and the results of such interaction, as well as the result of interaction between the gene and environment.

We have not fully considered the recognition of heterozygotes although this has been mentioned to some extent by certain speakers. The selective value of genes and the frequency of mutation for genes are not known as well as they should be.

Geneticists should look for new genes. I use that term loosely. There are genes we do not know anything about as yet. These may be discovered by some of the methods now available to us; one being the study of biochemical mutations. Some of these mutants cannot be considered abnormal, and do not cause nor are associated with abnormalities. They are discovered by studies of the urine and excretions. Investigators have been using paper chromatography in the studies, and have been able to determine that some individuals have substances consistently present in the urine. From preliminary genetic studies of families, investigators have noticed a tendency in some families for one or more children to excrete the same substance as does one of the parents, if the condition occurs in both generations. In some cases, both the parents but only some or none of the children have the trait, or the opposite conditions may be true. More studies of the biochemical traits are needed.

There should be some refinement of the techniques before paper chromatography can be widely used in genetic studies. Quantitative methods may be

[1] A discussion session of the Conference on Problems and Methods in Human Genetics, Bethesda, Md., October 8 and 9, 1953.

added to the studies. In a few cases microbiological tests have been used. Within time we can expect a series of genes to be discovered and analyzed genetically. They will be available for use in population genetics.

Human geneticists should know more about linkage. Some workers see little to be gained from information about linkage in man but there is some advantage in knowing more about our own genetic pattern. Also we have been slow in our study of human chromosomes. I do not mean that we can expect to find anything corresponding to the salivary chromosome in Drosophila but we may well get useful information from the chromosomal study.

What are the best methods we have for getting certain of the information we want? A study of the population can give us information about gene frequencies and possibly about changes in the frequencies. For these studies, will it be better to use a large population and study a few traits, or will we get more valuable information by using a small isolated population which is worked in considerable detail? I hope we will have some discussion of comparative advantages of the methods.

Several references have been made about the cooperation necessary for us to get the needed information in human genetics. With the possibility of opening up discussions of cooperation in research you might reconsider statements which have been made. I wonder which would be more advantageous to human genetics: to have a big unit, well supported, to carry on the research in a particular study, or to have several smaller groups, and sometimes individuals, working independently but in cooperation, and sometimes with an overlapping of efforts. That point will definitely arise one of these days when human geneticists go to foundations for funds in support of their research.

The study of our populations should be carried on. We must analyze for gene distributions in time and space, and study the effects of mutations and of selection for or against alleles. This information should be collected on our present population. Also we ought to attempt to predict what will happen in the future. It is time for us to consider applying the knowledge we have about genetics of man.

Those are some of the problems we should discuss although, of course, you may bring up any subject in which you are interested. Biochemical studies, linkage, the proper procedure to follow and cooperation necessary to get information we want, the best type of study to use with population groups, and the effects of genetic factors on the population in the present and in the future are topics for us to consider.

DR. F. C. FRASER, M.D. (*McGill University*): I would like to see a central clearinghouse for human genetics material. This might take the form of a Newsletter, like the Drosophila or mouse workers have, which would keep people informed about the unpublished projects of other workers in the field and would help to avoid so much overlapping of effort. Such a letter would also serve as a

medium for pooling information on rare conditions. I am sure many of us have a few cases each of a variety of rare diseases, but not enough of any one disease to analyze statistically. We all know the dangers of trying to combine data from single case reports in the literature. The only way to get around this is by combining data, properly collected (with due regard to the methods of ascertainment, etc.), of many workers.

For instance we have been fortunate in seeing 5 cases of the Ellis-van Creveld syndrome—as many as we can find in all the rest of the literature—I am sure several of you may have seen one or two cases. A News-letter could be used to circularize all the workers in the field. Then anyone who wished to could contribute data on his cases, which would be pooled with those of other workers, analyzed, and published under joint authorship or with some suitable form of acknowledgment. This sort of collaborative effort would also be useful for collecting series of twins with relatively rare diseases, where no one person can collect large enough numbers to be statistically reliable.

To do this successfully we would need (a) funds, perhaps supplied by an organization such as UNO or the U. S. Public Health Service, (b) a spirit of altruism and cooperation, which I feel would be forth-coming, and (c) someone willing to do the job.

DR. KEMP: An international organization for human genetics has been in existence through many years. It was established in London about 20 years ago, and it was called The Bureau of Human Heredity. In 1946 the board of directors (chairman: Prof. F. A. E. Crew) decided that the bureau and the material collected by the bureau should be transferred to Copenhagen and placed in the University Institute for Human Genetics. The bureau was moved in 1947, and the name was changed to the International Bureau of Human Genetics.

A provisional budget for the bureau was prepared and the minimal or basal annual expenses were calculated to $2,200. The bureau was affiliated to the Section of Human Genetics of the International Union of Biological Sciences connected with UNESCO. The bureau has served as a clearing house for material dealing with human heredity. Cases of rare hereditary human characters, abnormalities and diseases and pedigree- and twin-material on normal and pathological traits have been collected and studied. Material dealing with the subject of human heredity has been continued.

The activities of the Bureau of Human Heredity have been restrained, however, owing to the limited financial support obtained by the bureau until now. So far the bureau has only received an annual grant of $100 or $200 from the IUBS. There are, however, some possibilities that the support from IUBS will be increased in the future.

I should like to take this opportunity to tell something about my experiences as regards problems and methods in human genetics.

In Denmark the study and teaching of human and medical genetics devel-

oped in the Institute for General Pathology in Copenhagen in connection with cytological investigations and blood-group work. Lectures on human genetics were given to the medical students. This work was constantly growing, and in 1936 it was decided to separate the research work and the instruction given to medical students from the general pathology. This was the reason a new institute for human genetics at the University of Copenhagen was established. The Institute was built by the Rockefeller Foundation and inaugurated in 1938. The annual budget is paid by the Danish State through the University of Copenhagen, but the work is also supported by grants from several foundations and institutions. We have e.g. received generous support from the National Cancer Institute in Bethesda, Md.

Later the director of the new Institute was appointed professor of human genetics at the university and a member of the medical faculty and the medical students have as a part of their curriculum compulsory and facultative courses in general and medical genetics. The Institute has good cooperation with the Danish medical profession.

In 1938 the Institute was only an empty building, and no specific instruction was given to us as regards the way our work should be organized. We had to consider this question and realised that when our task was to study the hereditary diseases in the population we had to know and collect information about the patients suffering from serious hereditary diseases. This was the reason we established a Medico-Genetic Registry including a systematic registration, a card index, so far as possible complete, covering all the more serious hereditary affections observed in Denmark during recent years. In the Medico-Genetic Registry we have the starting-point for more thorough studies into particular fields, in many instances of the inheritance of a special lesion or disease. The procedure then is as follows: The physician, who is trained as a specialist in the field concerned, makes a thorough investigation of the individual patients with the disease or the lesion in question and of their families, partly on the basis of hospital records, other documentary material and genealogic investigations, partly by travelling about, visiting and examining the individual patients in their homes or by calling the patients to the Institute or to some hospital for observation and more thorough investigation.

Through the studies of various diseases and lesions, their mode of inheritance, their frequency and geographical or social distribution in the population, their etiology and pathogenesis, their clinical picture, the possibilities of their treatment and prevention, and the effective fertility of the affected have been investigated.

Besides these investigations of the inheritance of the individual diseases the institute is occupied also with other tasks such as, for instance, investigations of twins and of certain definite groups of the population, e.g. gypsies, prostitutes, some asocial elements, and adopted children. In this way the registration of the hereditary diseases in Denmark is supplemented.

On the basis of the experiences gained in these investigations, the Institute exercises its genetic-hygienic or eugenic activity as adviser on questions of sterilisation, induced abortion, marriage, adoption and of special relief. The material of the registration department has proved to be of much use to this activity.

In connection with the genetic-hygienic activity of the Institute it has been tried to follow and control the development and the fluctuation of the hereditary diseases in the Danish population.

This is a brief description of the methods we have used within the field of human genetics in Denmark during the last decades. In other countries other methods may be used corresponding to the local possibilities.

During the years after World War II a considerable development of the study in human genetics has taken place in Europe. Many new institutes, centers and university chairs for the study of human or medical genetics have been established, for instance England, France, The Netherlands, Italy, Switzerland and Scandinavia.

In U. S. A. and Canada the interest for human genetics has existed for many years. When I visited U. S. A. in 1932 I studied in Cold Spring Harbor with Dr. Davenport at the Carnegie Institution for Genetics and the Eugenics Records Office. The last mentioned institution does not exist any longer. Shortly after, Dr. F. J. Kallmann started his important genetic work at the Department of Psychiatry at Columbia University, New York. In Columbus, Ohio, Dr. Snyder also started investigations within the field of human genetics. Later new centers for human genetics developed, and after the war the American Society of Human Genetics editing the American Journal of Human Genetics was established. But when I heard about this conference on methods and problems in human genetics I considered it so important that I decided to come even if my time was rather occupied. Therefore I am very thankful for being invited.

SNYDER: I met Dr. Kemp first about 25 years ago at the Berlin Conference. Dr. Kemp is entirely too modest in saying that some of us may know about his publications. If there is anybody who does not know about his 20-odd volumes, he does not belong in human genetics. They are the finest publications we have in this field. We owe a great debt of gratitude to that group.

I wish to say something about the American Society of Human Genetics. For some years I was secretary-treasurer of the Genetics Society of America. In those days the secretary-treasurer did all the work—he was secretary, he was treasurer, he collected the bills, he ran the meetings. Now the Society of Human Genetics has a secretary, a treasurer, and a committee to run the meetings, and I am sure nobody has to do very much work. I don't see why they shouldn't do the type of thing suggested by Dr. Fraser. But it should be wider than the American Society of Human Genetics. It should be an international affair. I

think our M & G Study Section, with the help of the other sections of the NIH might very well receive a request for such an international service, and I think we should give it serious consideration. I am quite sure our study section would give sympathetic interest to such a secretariat on human genetics. I think this group might well come to such a conclusion and some group with the backing of the American Society of Human Genetics and other societies of human genetics might very well request some NIH support for such a secretariat to keep information flowing back and forth to many of us who do not know from day to day what other people are doing. It certainly can be done. This is the time—now—when the NIH has the interest in the field and, if I may say so, has the money to do it. I think we should do that sort of thing and I am perfectly sure we can get such a service that will keep each of us informed of what the other is doing. Some place where you can write in and ask questions and get answers to your questions. For example information about the hemophiliac woman. You can do it in Denmark, but we cannot in America. We do not know how you do it or how you arrive at that conclusion. I would like to see us get out of the rut we have gotten into and get onto an international avenue. I would like very much to ask Dr. Wilson right now if our group and other groups would not be interested in such a thing.

DR. J. WALTER WILSON (*Brown University*): I am not committing Mr. Eisenhower's money. I am a good New Hampshire Republican and I would answer with this. We have spent a lot of money in the past. I guarantee—although I retire from the USPHS a year from now—if you people come up with a project that will convince men like Dr. Snyder and the others that there is real scientific merit in it you will get support not only for one year, but 5 or 10 years. This is what the PHS is interested in—not in short-time projects but real things that will solve the big problems not only in medicine but in human welfare. This is what Mrs. Hobby wanted the Department to be in the first place,—the Department of Health, Education, and Welfare. This is where to begin—not in health or in education, but in long-time human welfare. If there is anything more important in this field than the problems in human genetics, I would be glad to hear about it.

DR. V. R. PHELPS (*Sparrow Hospital, Lansing, Mich.*): I wish to discuss an idea that I have been thinking about for some time. It has impressed me that, while the bibliography in the American Journal of Human Genetics is a clearing house for references in human genetics, there is so very much information that does not get into a journal. I am sure that each one here has had half a dozen or so cases which he has thought about publishing but decided that it was not worthwhile. Perhaps the records were incomplete. On the other hand, we have heard a lot here about breast cancer and certainly much information is being

obtained in those histories on topics other than breast cancer. Here we have all of these beautiful genetic records, so much additional data that we are gathering, going to waste. We are talking about it, yet we are not publishing it and may not—the principal reason being lack of journal space. The Annals of Eugenics in England publishes an appendix at the end. We cannot do it in this country for financial reasons. The A. S. H. G. is still running in the red on our new journal.

Also, I do not believe it would be possible or financially feasible in this country to have a central office for deposition of all our records. Even if centralization is not feasible it would be a help to know what information is available elsewhere, not only individual histories, but additional information on other studies.

If we could send a questionnaire each year to each person doing human genetics research in this country and ask how much information is being collected, we would know if a person in Michigan is collecting information on diabetes or somebody in Utah has data on cancer or a bone disease. I personally would be interested in knowing what persons have histories on bone diseases. If we had some such system, comparable to D.I.S. in Drosophila genetics, it would not be a financial burden. The records would be kept where they are, in the hands of the people who collected them. It would provide a method for gathering data and pooling information. Maybe we could make it really feasible so as to carry it on as a permanent project.

DR. MURPHY: I have been conducting a field study for the past 6 years. At the start of the investigation I needed a considerable amount of help in order to carry out the study efficiently. Unfortunately I did not have anyone to turn to for such assistance. Our greatest problem was the locating of all members of families. It would be a great service to those who plan future field surveys if the experience of those who already have conducted such studies could be pooled and be assembled in printed form.

PHELPS: When we talk about some place to write to, I think if we just knew we could find out where people were collecting data, we would be 5 years ahead, and let the institute idea wait. If we start too big I fear we would end up with nothing.

WILSON: Dr. Davenport had a eugenics laboratory that was going to do this sort of thing.

DR. KARL A. STILES, (*Michigan State College*): There was a mimeographed *Twin Bulletin* which first appeared in September 1941. This was assembled by Dr. Morton D. Schweitzer. It was the outgrowth of an informal meeting which

took place during the Philadelphia meetings of the AAAS in 1940. Requests for permission to use items in this publication had to be obtained from contributors. I regret to say that *Twin Bulletin* was a World War II casualty.

GOWEN: In considering an information service to report current progress, act as a repository for information which might otherwise be lost, make available technical notes, bibliography and directory of human geneticists, it seems desirable to consider other successful attempts. The Maize Genetics Cooperation News Letter was possibly the first in the field. It became a model for other groups as Drosophila Information Service, Mouse News Letter, Microbial Genetics, etc. All of these services were started by the groups primarily concerned. They were and are financed on a shoe string basis. To receive the current copy one must contribute something to it if no more than a letter saying that one has nothing to contribute to the particular number. This makes each service alive. Let's find some way to start, it would cost little save for postage and mimeographing, but start small so we can expand as need arises. The largest point is to keep the feeling of individual responsibility uppermost in the minds of those cooperating.

OLIVER: Has the thought crystallized to the point where you would like to move that action be taken on this, or do you want to hold it and keep it in mind and discuss it later?

STEINBERG: Perhaps it should be taken up at the Christmas meeting of the American Society of Human Genetics.

GOWEN: I think there is a good deal of interest here.

DR. W. E. HESTON (*National Cancer Institute*): With regard to the Mouse News Letter, I don't believe any formal action was taken. Someone offered to run it off on a mimeograph machine, and cards were mailed out and the information was sent in. Formal action may not be necessary now if someone who has a mimeograph machine will offer to collect the information and send it out.

PHELPS: I can do that.

OLIVER: Dr. Phelps, you will consider the possibility of doing this?

PHELPS: If I can get stamp money.

OLIVER: Now, Dr. Rae Phelps will do the best she can to get the news service started. If she calls on us for help, I hope we all respond. Before Dr. Phelps

leaves to catch the plane, she might want to tell us how she plans to handle the information we send her.

PHELPS: Any ideas anyone has will be greatly appreciated. I wonder what you would think of a mimeographed form-letter containing an explanation as to why this information is being asked, to be sent to all members of the American Society of Human Genetics and then to all people we can contact who might be collecting material of this type. We will need to set up a roster of names and addresses. You will all need to help in contributing names. You may know of some person who is interested in collecting records but is not a member of the society.

Then we will need to standardize the way of reporting the records available. I don't know how you might feel about listing the number of records. Some may say, "I have one isolated case on which there is, however, a great deal of information and it should be listed". It would be very desirable to know something other than that the pedigrees were simply ones of muscular dystrophy or breast cancer, i.e. what data pertain directly to the diagnosis of the condition, what additional information is obtained, or perhaps that the study is one done in an heredity class where students fill out questionnaires on their own families. Are questions asked about diabetes, heart trouble, and other things in the family? We need some abbreviated method of showing these things in the record. We should have available all of the names of the investigators associated on the project and the location (individual, institute or clinic) of the records.

DR. ALLEN: I should like to say that one thing wrong with some bulletins like D.I.S. is that they encourage the broadcasting of premature ideas or unverified findings, which are better restricted to personal contacts. Sometimes, too, valuable bits of information fail to get into permanent literature because the author who has placed a note in one of these bulletins may feel less incentive to write it up for a journal. I think that, in planning a bulletin for research workers in human genetics, we should confine the subject matter to types of data available and topics under study.

OLIVER: The problem I now present is not too closely related to the "center" we were discussing. It has to do with an idea which Dr. Kemp brought to your attention and one Dr. Snyder referred to last night. It is the problem of a shifting population and the addition of deleterious genes to the population—and what effect that might have on populations of the future. I would like to open this topic for discussion before we consider other points.

MR. ROBERT COOK (*Journal of Heredity*): I got into this "act" by being asked to discuss lethal genes before the International Congress on Fertility and

Sterility last May. In the strict sense lethal genes cause reduced fertility rather than sterility. Sterility genes are not lethal in the strict genetic sense, but their effect is similar. I called attention in this discussion to Dr. H. J. Muller's presidential address before the American Society of Human Genetics, presented on December 29, 1949, in New York. There Muller raised the question of the possibility that a rapid build-up of deleterious mutations—lethal or morbid—may be taking place in our population because of the shifting pattern of mortality which has followed the vital revolution instituted by Jenner, Pasteur, et al. This paper stimulated the interest of a person who has long been concerned with the problem of human genetics. He raised the question as to whether evidence exists of such a build-up in morbid genes and, if the evidence does not exist, he wished to know what steps might be taken to settle the question. He also wished to know what might be the rate of reduction in overall fitness (or viability) which might be expected to result from an accumulation of such genes.

I sent out a rather hastily prepared mimeographed statement to a few of those who were scheduled to attend this meeting. There has been considerable interest expressed in these questions. There has been a sharp division of opinion as to whether such questions could—or should be asked—and as to what steps might be taken to gain useful information regarding the points raised. I am sorry to learn that Dr. Snyder has had to leave—he represented the "con" view rather strongly: The view that this matter could not usefully be inquired into.

A specific point has been raised, which might serve to get the discussion going. If recent changes in mortality do represent a considerable reduction in selective pressure against morbid genes, may not that be a good thing? The best way to speed evolutionary change so this argument goes is to increase variability. I would like to hear from Dr. Allen on that and perhaps Dr. Jay Lush will be interested in commenting on that point of view.

DR. ALLEN: There must be others here who could make this point, and defend it, better than I. It seems clear to me that relaxation of selection must usually have a tendency to promote evolutionary advance. I believe geological history gives evidence suggesting a relaxation of selective forces at several points where large evolutionary advances occurred, such as the sudden diversification of mammals when the large carnivorous reptiles became extinct.

If the relaxation is temporary, the species may develop some valuable, but previously impossible genotypes before selection is resumed. If the reduction of selection is permanent, it can be so only with respect to certain traits, and other traits, still important for survival, will then receive the full effect of mutation and natural selection. For example, if susceptibility to minor bacterial infections becomes a harmless trait, then genotypes producing such susceptibility will become more numerous and more varied. If some of the genes

involved also produce major susceptibility, selection will not cease to operate against those particular genes. At the same time, if any of the new genotypes result in a previously impossible mechanism, for example a new mechanism of immunity or of brain function, the average of the species will be improved and a new avenue may be opened for further evolution.

LUSH: I don't think I have worked on that at all. Anything I say would be repeating my understanding of some of the ideas Dr. Wright has expressed; namely that when we are up against some kinds of epistatic setups, relaxation of selection permits (but does not impel) advance through avenues otherwise closed. It would take hundreds of thousands of generations for unaided mutation to produce much change. But we are immediately confronted with the fact that most genes have many pleiotropic effects. Relaxation of selection for one effect is unlikely to be relaxation of selection for all effects. Usually it merely shifts the net selection coefficient for that gene to some other value which is not often zero.

OLIVER: Does anybody feel as strongly about this as does Dr. Snyder?

DAVID: I would not presume to speak for Dr. Snyder; however, I will presume to speak for myself, and knowing his viewpoint, I think he would be, to a reasonable degree, in agreement with me. The document that Cook circulated, and the covering letter, include sections that I found very disturbing. I'm not going to try to discuss the underlying theoretical considerations. I have read Muller's papers on our load of mutations rather carefully, and Dr. Snyder has read them. We think we have found some basic fallacies in his argument. The question is briefly discussed in a chapter Dr. Snyder and I contributed to Leavell & Clarke's "Textbook of Preventive Medicine." At the moment, I will comment only on the portions of Mr. Cook's communication that I found particularly disturbing, and perhaps others will have something to add on the theoretical side.

Item one is the following paragraph, which seems to reflect a sense of values that I find almost incomprehensible: After reviewing the Muller argument, Mr. Cook writes: "Obviously, this situation poses some very important practical problems. In the long run, the effect of suspending natural selection could be more prejudicial to the survival and progress of the human race even than atomic warfare—which would—of course—speed up the process of adaptive degradation." In my view, the urgency and magnitude of the immediate threat to the human species implied in the possibility of atomic warfare hardly permits the remote and contingent dangers suggested by the idea of genic erosion to be mentioned in the same breath. I cannot forbear from adverse comment on the sense of values which the paragraph quoted seems to imply.

In the covering letter, the question is asked: "If you agree that the basic

postulates . . . . are sound, what do you consider the most important steps to take first? What pilot studies do you consider would be best calculated to establish the evidence that would make the case so convincing as to stimulate an all-out, broadly based, attack on the problem?" The thesis, as I read this, appears to be accepted already, and there remains only the problem of finding the best way of substantiating it.

Finally, Mr. Cook adds that an attack on this problem "would also involve an educational campaign to present to the medical profession and to the public at large the nature of this creeping crisis." Certainly there are aspects of the problem posed by Mr. Cook which merit scientific investigation; such investigation, in fact, is in progress in several competent hands. But I would object strenuously if it were felt necessary to seek support for these studies by raising the cry of a "creeping crisis," the seriousness of which—and indeed its very actuality—is at best debatable.

DEMPSTER: In this field we are certainly entering a very speculative area capable of containing a variety of non-disprovable opinions. In rather blithely discussing the genotype of the future, however, is it perhaps possible that many genetists have insufficiently reflected on the cost of arriving where we are today? Natural selection must have been a stringent and brutal process to have produced, or even preserved, the remarkable average degree of perfection in all organs and physiological functions that we observe today. The involuntary sacrifices that have contributed to the present germ plasm constitute an immense investment, and for the long term it is certainly proper to be concerned with a possible needless dissipation of this accumulated capital; recovery of losses might command a heavy price from future generations.

If there were a fuller realization of the cost and value of this genotypic capital, perhaps attitudes would be more critical toward many arguments whose chief claims to acceptance, it appears to me, must be their bolstering of previously formed convictions. In particular I can see no other basis for accepting, as support for a deliberately planned relaxation of selection, the argument that the mutations whose accumulation would be thus facilitated, even though currently harmful, might eventually enter into a multiplicity of new and beneficial epistatic combinations subject to future selection, thus accelerating the evolution of man. Perhaps deceleration would be much better after all; but supposing the shifts of average genotype should be aided and abetted, is genetic plasticity so lacking in man as to force the adoption of desperate and unlikely remedies?

I am not attempting at this time to argue either against all artificial selection plans nor in favor of any one of them, although my personal opinion is that some small beginnings in a voluntary program may even now be possible with respect to known flagrantly deleterious genes. My plea is for more conscious

efforts toward examining arguments on their own merits irrespective of the conclusions to which they lead. There are certainly many genuine, although not necessarily conclusive, arguments in favor of a laissez faire or nearly laissez faire policy at the present juncture, among which might be mentioned our ignorance of human population genetics and the consequent probability of errors in aims or means, special difficulties due to social stratification, the harm that could result from stimulating undue enthusiasm among the genetically uninformed, and the impelling necessity of more immediate problems. But we are, I believe, only deceiving ourselves and others if we claim to know that the changes and relaxations of selection characteristic of modern civilization will leave man, or lead him, genetically where we would like him to be.

STEINBERG: I would like to make a comment at a much lower level of sophistication. One of the first points raised at the end of the mimeographed memorandum from Mr. Cook is, how rapid is the build-up of deleterious genes. I stopped right there and considered what is a deleterious gene. In elementary genetics, I learned that the action of a gene is conditioned by the residual genotype and the environment. I learned that we do not have deleterious genes, but deleterious genotypes and that these must be considered in the environment in which we find them. If the environment is such that the genotype survives, the genotype, it seems to me, is no longer deleterious. Many genotypes which were formerly deleterious are no longer so because of the work of physicians who have managed to make them "fit" by changing the environment. It seems to me that we no longer have a problem as far as these genotypes are concerned.

I address myself to Dr. Dempster's point. Perhaps there is a problem, but after all successful medicine has been going on for a very limited time relative to the time period for the evolution of man. Human genetics has been going on for a still more limited time. Suppose we delay our worry for 100 years, slightly over three generations of human beings. I think all of us are willing to concede that no genetic catastrophy is going to happen to the human race in three generations. By that time, we may know a little more about the problem. I would rather see us devote our time, energy and money to learning more about human genetics than to worrying about a long range problem which we are not equipped to tackle and which may never arise.

GLASS: What I had wanted to say has been rather well stated by Dr. Steinberg. One further word—that which relates this discussion to the questions raised earlier this evening about what happened to human genetics some 20 years ago when, as was said, the fanatic views of the eugenicists ran the thing into the ground. As a young student 21 years ago, I was at the meeting of the Eugenics Society in New York when Muller made that speech to them on the dominance of economics over eugenics which created quite an uproar and just about fin-

ished the activity of the Eugenics Society. Now it seems to me that the tables are a bit reversed. This view of Muller's that is being discussed is a theory of the change in gene frequencies that he believes to to be occurring, and he has introduced certain calculations to support that view. But the question is still: is it a fact or not? I think we have to bear in mind that we must not adopt a thesis and then go out to prove it. What we have got to do is to keep our feet on the ground and set to work to study gene frequencies in populations; to determine whether they are decreasing or increasing, irrespective of whether we think these genes are deleterious or not. When we know more about what happens to gene frequencies in populations, then we can test the ideas that have been advanced and see whether they hold water or not. First let us find out what happens to frequencies in populations.

DR. HERNDON: Although I agree with the previous speakers who suggest that we need not get hysterical or jump to conclusions without adequate data, I do not feel that this problem can be ignored or postponed. Medicine has made tremendous strides in preventing deaths and alleviating disease processes in the past century, most of the improvement in mortality being in the younger age groups. This process certainly has relaxed forces of natural selection and must have some effect on the frequencies of deleterious genes. The fact that many generations would probably be required before any foreseeable shifting of gene frequencies can have any marked effects on our population should not relieve us of the responsibility of facing the problem at present. Basically this is a public health problem involving the future health of the nation. In order to detect any trends it is necessary to have at least two points on a graph. Dr. Steinberg suggests that we might well wait 100 years before facing the problem without catastrophic results. I feel that we should attempt to determine one point on the graph now, and then let Dr. Steinberg determine the second point in 100 years. I feel that we are in need of extensive gene frequency studies now so that an accurate basis of comparison will be available in the future.

DEMPSTER: I am very much in agreement with the last speaker and especially in agreement with some of the points made by Dr. Glass. On the other hand I object to the argument that states we should do nothing now because we *know* it is not necessary or because we *know* it will be to our genetic advantage to do nothing. Do not let us lead people to believe things of which we ourselves are very ignorant and about which we in fact may have reason to feel very dubious. A related argument that appears in the printed writings of geneticists runs something like this: selection in a multigenic situation cannot possibly make appreciable headway except over a period of hundreds of generations. I know of no evidence from experiments with domestic or laboratory animals that offers support to such a contention.

DAVID: In spite of the fact that I am second to none in my esteem for Dr. Muller, I think he has jumped the gun in accepting as virtually an established fact, or if not an established fact an established theory, a thesis which to my mind is extremely dubious.

I think Dr. Herndon raised the question of whether we were becoming more fit or less fit because of public health progress. All data known to me say clearly that we have become more fit. Fitness, of course, involves a reciprocal relation between genotype and environment. In the present state of our knowledge, it seems to me rational that our emphasis should be on attempting to provide environments in which genotypes currently available can achieve optimal expression, rather than on trying to plan a distribution of genotypes optimal for some future environment the nature of which we probably cannot even imagine. This emphasis would obviously call for continued progress in medicine and public health.

There is a point involved here which is pertinent to Dr. Muller's argument. Muller's thesis, if I understand it correctly, is that if you reduce the selective pressure operating against a given genotype, the frequency of the genotype will tend to increase to a new equilibrium value and the aggregate damage attributable to the genotype will be the same as it was at equilibrium frequency before the selective pressure was reduced. I believe that this is unassailable in so far as you can trust theoretical considerations in this area at all. Our public health and medical measures then are, on his theory, not worsening the situation, unless something happens to speed up mutation rates. Meanwhile, there is one important class of exceptions to this general rule that the total damage attributable to a gene with deleterious effects remains constant under changed selection pressures, and this is the limiting case when the deleterious effects of a gene are completely erased. The average damage done by the gene has now become zero, regardless of its original value.

OLIVER: We still have several questions to discuss. One of them I think is worthy of consideration. The one I refer to has to do with possible new methods for finding genes which we can use particularly in our studies of linkage or with any of the combinations we want to make. I refer to chromotography and physiological responses. If any one has an opinion he would like to express on this matter, I believe it will be worth while. These are methods that more and more of us will want to use. I believe that some one said today he does not know much about chromotography and the use of that method for testing for variant types. I also believe that Dr. Steinberg said he has some information about another method of study he believes is good.

STEINBERG: My information is minimal—almost limited to what Dr. Oliver has mentioned. An Englishman spoke at the medical center—I am sorry I don't

remember his name, but he is the man who developed the system of paper electrophoresis. He described the method and illustrated its use. We were all impressed with the cheapness and the rapidity, accuracy and reproducibility of the results not only qualitatively, but quantitatively. He used this technique to isolate Castle's intrinsic factor in pernicious anemia. The apparatus is used in his hospital as a routine aid in diagnosis. It measures the different proportions of the protein fractions of blood plasma which are found to vary from disease to disease. The basic apparatus is easy to construct and to use; to quote him, "Even a surgeon can and does manage it."

WILSON: I have an urge to say a word here, particularly because of this discussion of paper electrophoresis. It is a technique that has great promise. I am sorry that Dr. Neel is not here as he has outlined in beautiful style a study he has in mind comparing American and African negroes. Electrophoresis is only a tool, but the thing that is symbolic to me is that the increasing use of such tools indicates that genetics is progressing to a really sound scientific level. The work that Dr. Macklin has so patiently done shows that human geneticists are willing to undertake long-time programs where a momentary result is not expected, but which will ultimately produce answers of real scientific value. By use of electrophoresis we may get quicker answers to some things.

OLIVER: We need more genetic material. I see possible value from the use of new methods, particularly if they can give us more useful known genes to use along with the genes for blood groups and other types in studies of linkage.

STILES: Paper chromatographic methods may provide the human geneticists with a very useful tool. Paper chromatography may make new advances possible in physiological genetics of man. One of the limitations of chromatography at present is that it has little value for quantitative studies, however, new methods are being developed which show great promise.

Geneticists may find it necessary to use chromatographic techniques to first carry out investigations that are purely biochemical in an effort to pave the way for genetical studies in which they are primarily interested.

OLIVER: As I mentioned, they definitely do find differences within the family and it is suggested, at least, that there is an hereditary tendency for excess excretion of certain of these amino acids.

SPUHLER: The paper chromatography, work at the Institute of Human Biology is under the direction of Dr. H. E. Sutton. In its biochemical studies, the Institute is concerned with three main objectives: (1) to establish the range of biochemical variability of about 50 items, both among and within individuals

(2) to determine the extent of hereditary control of the biochemical variables by means of twin studies, (3) to study the mode of inheritance of those substances which have been shown to be under genetic control. The measurements made thus far have been on blood, saliva, and urine. In addition to some of the standard colorimetric tests, paper chromatography has been used extensively to measure amino acids and other detectable substances. Future plans also include the measurements of activities of various enzymes. I think the work at Michigan is more genetically oriented than the pioneer work by Dr. Williams' group at Texas.

OLIVER: They have done some work in genetics. Of course there are not enough big family groups. The work has been with small families, trying to get the program established.

DR. MACKLIN: May I suggest that in the future, when grants are being made for such studies as these discussed tonight, that the granting agency, without attempting to dictate the method of approach to the problem, inform the applicant that help in the design of his experiment, should he desire it, is available. The group which Dr. Lilienfeld has mentioned as being useful for controls in such a study as mine, might be used by workers in that state; controls drawn from that might be worse than useless for a project being run in another part of the country. I applied for assistance before I began my project to an expert in the design of experiment, and had his advice. Apparently different experts in the field have different ideas about what should go into such a study. If such advice were offered, there might be less criticism later.

GLASS: I would like to suggest an answer to Dr. Macklin's question how this can be done. The blame is not to be laid at the feet of the granting agency.

DR. MACKLIN: I am not blaming them. I am suggesting that they might consider this policy.

GLASS: In the State of Delaware at the present time there is being conducted a very extensive survey based on a random sampling of the Delaware population, in order to determine the frequency of mental retardation of all kinds. It is not supported by the PHS but by another branch of the Government; and it is a very large and expensive project organized by people like the rest of us who have developed an idea about what should be done and how to do it, and who presented their case well. On this particular project there is an advisory committee—I don't know whether it was required by the granting agency or whether it was the idea of the investigators themselves, but anyway there is an advisory committee. The people running the project are essentially psycholo-

gists and psychiatrists. The advisory committee consists of a statistician, a sociologist, psychologists and psychiatrists from elsewhere, and a human geneticist, so as to make up a variety of people who can give advice about specialized aspects of the study and about the things they thought ought to be included in the project. I think that when we apply for our grants and undertake to plan our programs, we ought to think about the possibility of including requests for advice of that kind, and very likely we will be heard.

WILSON: It has been one of the policies of the U.S. Public Health Service that it never will try to tell an investigator how to do his investigation, but he can get advice from the Public Health Service if he wants it. I think Dr. Meader will back me up on this statement.

DR. RALPH G. MEADER: (*National Cancer Institute*): That is the basic theory.

DR. MACKLIN: Perhaps the person asking for the grant does not realize that this help is available. I agree that there should be no group supervising the research; dictating what the research worker should do. This takes away all individuality from the work and the worker. Actually, the very fact that the data on all the projects are not collected in the same way is an advantage. It enables the workers to sift out what is good from what has sources of error. The diversity of approach is desirable. Moreover, the advice which the research worker receives may not be the best advice, since although the advisor may know a great deal on some aspects of the subject he may not know other aspects as well as the research worker himself knows them. I still feel, however, that a statement as to a source of help might be desirable.

WILSON: You understand the difference. There is always the accusation possible that "he who pays the piper plays the tune." It is perfectly true that the granting agencies do not have the answers either. It is part of the researcher's job to find out where these are.

MEADER: Although there is this basic philosophy of trying not to interfere with the ideas, hopes, and aspirations and opportunities for success or failure that the investigator has, because that is part of the scientific method, there is nevertheless a strong feeling, at least on the part of one granting agency, that it would like to be of aid and use wherever possible. There are limitations so far as our own personnel are concerned. Obviously we cannot have enough people on hand to service any program that needs biometrical advice but, within the limitation of time that our people have, they are anxious to serve and have served in that capacity to grantees.

For you who are undertaking problems needing biometrical advice, there is

another part of our granting organization that is of service. There is a Committee on Standards for Grants Surveys of the Public Health Service. This was more or less forced upon us by the Bureau of the Budget, and it came about in a very peculiar fashion. During the war or earlier some of the agencies of the Government were sending out questionnaires of various types to all types of business people to collect data of one type or another. The business people complained about the paper work, and after study an Executive Order was issued which restricted the sending out of such interviewing material unless it had been cleared by the Bureau of the Budget which was supposed to supply some sort of advice and control. The Public Health Service was disturbed by the possible interference if this were extended to grants for problems of interest in the public health field, and so they secured permission to set up their own Committee on Standards.

Any project which is to undertake the interviewing of a general group of individuals—this does not apply to a doctor referring to his patient or within a hospital group—and for which a Public Health Service grant is to be made would have to be submitted to the Committee on Standards for review as to the propriety of the undertaking, the amount of interference with the normal operation of citizens that the matter would entail, and as to the adequacy of its method. That committee includes some biometricians and statisticians, as well as others whose qualifications I can't cite to you at the moment. That committee does perform that function and it was not in existence when you started, Dr. Macklin. I don't believe that very many of us would have felt earlier that it was proper or that we were necessarily qualified to tell you what sort of controls you should pick or how you should pick them. We would assume that you had greater knowledge.

Of course, I still think that the ideal situation, as someone has suggested, is that a person who is going to undertake this type of study might be wise enough to realize his shortcomings, and get the help he needs. The question comes often, who do I know who is competent to advise me. I understand that biometricians do not all agree as to what are the proper methods. There is then the problem of selecting a competent biometrician. It is like saying, how do I pick out my doctor. Perhaps we need to set up some sort of standards for statisticians and biometricians that will guarantee they are 99 and 44/100's percent pure in their capacity to be of aid. I do not want anyone to think this is a reflection on the biometricians in general, however.

COMSTOCK: I want to make an additional point as a statistician. It is much easier to criticize the conduct of work that has been done than to provide sound advice on procedures for use in future work. The obvious reason is that in completed work many sources of bias and inaccuracy need only be recognized whereas with respect to future work they must be predicted and ways

devised to minimize their ill effects. It may be that statisticians are too prone to criticize conduct of completed investigations. This I do not wish to argue. It is one way to emphasize issues that may also be pertinent in plans for new work; perhaps it is overdone. The point I wish to make is that serious statisticians with some experience that I am acquainted with are very cognizant of the fact that sound advice on statistical issues requires intimate and detailed acquaintance with the problem, related issues, and limitations within which the research worker must operate. As a result you must not expect such statisticians to be willing to advise on procedure unless you have afforded them the opportunity of thorough acquaintance with your problems. Otherwise the most you can expect is that this or that procedure may be good with no guarantee attached to the actual value of the suggestion.

DR J. H. BENNETT (*Carnegie Corporation*): It is pleasing to see in this conference an expression of the widespread scientific interest being taken in human genetics in the United States. The geneticist visiting Northern America is impressed by other more visible signs of this same interest, namely the growth of the numerous centers for the study of human genetics. The work of these centers with their different characteristics is being followed keenly overseas. At the University of Melbourne where financial provision has recently been made from private sources for studies to be undertaken in human heredity, we shall enjoy the benefit of the experience of these American centers and of a familiarity with the ways in which they have tackled the problems by which they have been confronted.

DR. ALLEN: Dr. Sheldon Reed says that each year the Dight Institute learns of a certain number of brother-sister matings, and these are reported to them before the babies are born. As he points out, such material should be nearly free of bias with respect to congenital abnormalities, and in sufficient quantity it might give much information on the frequency of deleterious recessive traits in the population. If anyone else can obtain similar cases. I believe Dr. Reed would like to hear about it, and a systematic collection of the data should be quite rewarding.

WILSON: I want to say first of all that I sat through two very satisfactory days, as far as I am concerned, and I want to compliment Dr. Oliver, whose baby this was as I told you the first day, on having done this job. I also wish to thank Dr. Heston, who has done most of the work although he denies it, and who has put a lot into the job. I hope we have done something and I am looking forward to the time when, as Dr. Herndon says, Dr. Steinberg will come back 100 years from now and find out what we accomplished.

BOOK REVIEWS

La Malattia Emolitica Del Neonato

By R. Ceppelini, S. Nasso, and F. Tecilazich, Milan: Istituto Sieroterapico Milanese Serafine Belfanti, 1952, pp. 389

This book presents a clear and readable account of hemolytic diseases of the newborn and the modern blood group work which has done so much to explain these disorders. The language is simple, yet the explanations do not lack in detail in any way and are thoroughly up-to-date.

The authors begin with general considerations of blood antigens and the genetics of such factors, considering them as examples of polymorphism in man. The various types of antibodies involved are thoroughly discussed and clearly explained. Special emphasis (67 pages) is given to the Rh system, but good discussions are also given of the ABO system, the MNS system, and others, including P, Lutheran, Kell-Cellano, Duffy, Lewis and secretion factors, Kidd, Jay, and rare antigens.

The second part of the book is devoted to etiology, pathogenesis and nosology, and the third part to diagnosis, prophylaxis and therapy. The fourth part, 39 pages, is devoted to technique.

The discussions of the genetical basis of the blood groups are especially good. In the Rh system, both the Wiener and the Fisher-Race notations are used, with no attempt to decide which is better. In the body of the text the authors show a distinct tendency to prefer the Fisher-Race notation.

Although no advanced mathematics is used, the discussions of the calculations of gene frequencies and the use of statistical methods are very good.

This book should form an excellent simply written introduction to the study of hemolytic disease of the newborn and to modern blood group work for all who can read Italian.

WILLIAM C. BOYD
Boston University

Race Crossing in Man (Eugenics Lab. Mem. XXXVI)

By J. C. Trevor, New York: Cambridge University Press, 1953, Pp. 45, $2.50

This brief monograph is a mixed blessing. On one hand it demonstrates what a wealth of metrical material has been collected on human hybrid populations, while on the other it presents methods of analysis which are both inefficient and ineptly applied.

In his preferatory note, Dr. L. S. Penrose points out that this work was done prior to the start of hostilities in 1939, but that the value of the data presented is timeless. This is very true, however the analysis made of the data is rapidly showing signs of age. The data are a compilation of published records of nine outstanding cases of biracial crossing; Hybrid American Negroes, Jamaican 'Browns', Half-Blood Sioux, Ojibwa-Whites, Yucatecans, Rehoboth Bastaards, Kisar Mestizos, Norfolk Islanders, and Anglo-Indians. The mean and standard error are recorded for stature and seven cranial measures for most of these populations. Additional measurements are noted for many. In each population studied the

sample includes 25 or more adult (20 years or over) individuals of each sex. The values for each sex are recorded separately.

The mean of the hybrid population is compared with that of each of its propositus population groups by the use of Student's t test. Unfortunately this test requires the assumption that the variances of the populations being compared be the same. Nowhere in the presentation is this recognized. It would have been eminently desirable to determine the significance of the variance ratio for each parameter for each pair of populations compared before the t test was applied. For cases of significant difference in variance between the populations the Fisher-Behrens method for the use of t with samples of unequal variance would be applicable.

The variability of the propositus and hybrid populations is considered separately by the method of Mourant in which the variance ratio of each character for each population pair is found and then the mean variance ratio for each pair determined. This analysis indicated that the variance of the hybrid population is greater, but was found, by a t test, to be significantly greater in only two cases. Here it would have seemed desirable to look up the values in a table of F to get a more powerful estimate of the difference between these populations.

The material presented in this monograph provides a good addition to the blood group, dermatoglyphic, and taster frequency data which are currently used in the analysis of population dynamics, and should serve to attract the attention of interested workers to this relatively undeveloped body of information.

KENNETH S. BROWN
University of Chicago

REPORT OF THE SECRETARY

A. MEMBERSHIP AND SUBSCRIBERS TO THE JOURNAL

The membership drive continued to be successful because of the loyalty and cooperation of the members of the Society. The future of the Society depends upon the number of individual members who are willing to take the small amount of trouble involved in nominating one or more persons for election to membership. During the past year 170 new nominations and institutional subscriptions were obtained, a very substantial gain, which brings us within about 100 subscriptions of the "break-even" point of self-support for the Journal.

The statistics as of September 1, 1953, follow:

Patrons	3
Life Members	3
Active Members	462
Associate Members	14
Corresponding Members	45
Individual, non-member subscriptions	21
Institutional subscriptions:	
Foreign	78
Domestic	212
Total	838

The fourteen associate members do not receive the Journal and there are 66 suspensions, leaving 758 paid subscriptions.

Last year there were 118 members or institutions in arrears compared with only 66 this year. The treasurer, Nash Herndon, M.D., deserves a vote of thanks for this improvement in collection of the annual dues.

B. NOMINATIONS AND ELECTIONS

The nominating committee was composed of W. E. Heston, H. F. Falls, M.D., and A. H. Hersh. The following persons were elected for duty:

For one year terms

President	James V. Neel, M.D.
Vice President	A. F. Blakeslee
President-elect	Curt Stern

For two year terms

Directors at large—Ray C. Anderson, M.D.
H. Bentley Glass
A. G. Steinberg

C. THE BOSTON MEETINGS

The meetings were held in the Sheraton Plaza Hotel, Boston, Massachusetts, December 27–30, 1953. It is the impression of the secretary that the program was well balanced

and composed of excellent papers. The three symposia were very well attended, two of them being joint symposia with other organizations but initiated by our Society.

The dinner and address by the President were attended by 115 persons.

The next meetings will be in Gainesville, Florida, September 5–9, 1954. We have been assured that Gainesville has excellent facilities, including air conditioning. September is a more satisfactory meeting time than December for most members.

Respectfully submitted,
SHELDON C. REED
Secretary

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